

KNOWLEDGE TO ACTION IN THE ANTHROPOCENE:
UNDERSTANDING AND MANAGING BIOGEOCHEMICAL CYCLES
UNDER ANTHROPOGENIC GLOBAL CHANGE

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Abstract

Through rapid and persistent anthropogenic global change, we now recognize that human activities risk fundamentally altering the functioning of the earth system, with potentially devastating consequences for human livelihoods. Rapid environmental change has led to growing interest in the development of new governance approaches to manage the biogeochemical dimensions of these challenges, from carbon and sustainability accounting to the use of ecosystem service valuation in decision-making. Yet our understanding of what makes such approaches successful and what kinds and forms of scientific information are needed to inform them is still nascent. My dissertation seeks to inform this understanding, asking: What are the conditions under which governance approaches to the management of human alterations of the carbon and nitrogen cycles work best, and what social, technical, and institutional barriers exist to successful adaptive approaches to address the challenges of the Anthropocene? Using a variety of methodological approaches, ranging from controlled experimental designs to social scientific studies of conservation decision-making, I essay to contribute to our understanding of sustainability science both by producing new, management-relevant scientific knowledge about anthropogenic global change, and by simultaneously critiquing and crafting new and emergent forms of environmental decision-making.

In my first two chapters, I use observational and experimental approaches to understand how carbon cycling responds to a variety of anthropogenic stressors at different scales in two different ecosystems - an Arctic coastal sea and a California grassland - with an eye toward the incorporation of such information into ecosystem management frameworks and earth system modeling. In my next two chapters, I assess

the treatment of one particular ecosystem service – carbon sequestration in coastal ecosystems – in two specific governance contexts: the implementation of the National Environmental Policy Act and the work and missions of coastal conservation organizations. The results of this research show that ecosystem carbon storage is currently viewed as an implicit offset that can be used when it is convenient to mitigate other greenhouse gas emissions. The results also highlight that even where there are win-wins between habitat conservation and climate mitigation, there exists local-scale resistance to commodification of ecosystem services when storing carbon doesn't align with a conservation mission built from a sense of place. Both of these results have implications for our understanding of what can make adaptive environmental management work in the political, ecological and social contexts of rapid global change, as well as for the development of future guidance on environmental impact accounting. My final two chapters focus on a case study of water quality management in the San Francisco Bay estuary. The results from both experimental and observational studies challenge existing literature on the ecological consequences of wastewater discharge, showing that high levels of ammonium (NH_4^+) loading are not the cause of declines in ecosystem production. As such, they directly inform both our understanding of phytoplankton ecology in human-impacted estuaries and the development of new metrics and indicators to assess and control anthropogenic impacts on estuarine water quality.

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Introduction

The Anthropocene: Anthropogenic Alteration of Carbon and Nitrogen Cycling

Anthropogenic global change is rapidly altering earth system functioning, with significant consequences for organisms, ecosystems, and human livelihoods across the planet (Steffen et al. 2007). Recent assessments of these changes have outlined “planetary boundaries,” – magnitudes of anthropogenic disruption, that, if maintained, will lead to fundamental and irreversible changes in earth system functioning (Rockström et al. 2009). Rates of anthropogenic biodiversity loss, greenhouse gas emissions to the atmosphere, and increases in fixed nitrogen delivery to ecosystems are all currently understood to exceed these boundaries (Rockström et al. 2009).

The *Anthropocene* refers to the idea that humanity currently exists in an era in which earth system functioning is inextricably structured by the activities of human beings (Crutzen 2002, 2006). Many biophysical scientists have embraced the idea of the Anthropocene as a term describing the current geologic age with utility for understanding biogeochemical cycles that exhibit anthropogenic influences (Codispoti et al. 2001), as a wake-up call to the potential for significant societal disruption from these changes (Steffen et al. 2011), and as an opportunity to pivot scientific discourse toward directed planetary stewardship, through sustainability science (Steffen et al. 2015).

Some social scientists have regarded the Anthropocene concept with suspicion (Sayre 2012), as a cover-up for failures to address chronic failures of environmental policy. Others have linked the concept directly to inequalities in underlying political and economic structures (Lovbrand et al. 2009), expanding its scope of scholarly

application into the social sciences and humanities. Other social scientists have embraced the defining of the Anthropocene as an opportunity to transform the narratives of sustainability to include the human-dimensions of these problems (Biermann et al. 2012). They contend that in a human-dominated, synthetic world, we can cast aside the environmentalist movement's reliance on narratives of the environment as an "other" being harmed by human activities (Latour et al. 2011), and can, instead, recognize that environmental problems are also problems of society.

Emergent Institutions for Biogeochemical Management

At the heart of many of these discussions about what we can do to address the sustainability challenges of the Anthropocene are fundamental tensions about how to define – and thereby measure and mitigate – that which constitutes a "human impact on the environment." Alongside the rapid ecological changes in the global carbon and nitrogen cycles that have recently been documented, the last two decades have also witnessed a proliferation of new governance frameworks for addressing these impacts. In particular, in response to increasing appreciation of the harms of climate change and the need for mitigation actions, practices of carbon and sustainability accounting have rapidly developed (Ascui and Lovell 2011). From individual carbon footprint calculators (Wiedmann and Minx 2008), to accounting of "Scope 1, Scope 2 and Scope 3" greenhouse gas emissions (Hillman and Ramaswami 2010), and from nutrient trading frameworks to fully-implemented cap and trade programs and carbon taxes, to methodologies for national greenhouse gas inventories submitted to the United Nations Framework Convention on Climate Change, the rate of development

of new rules and regulations of biogeochemical cycles for sustainability has been exponential over the last two decades.

Some 25% of global anthropogenic GHG emissions come from sources other than the combustion of fossil fuels (IPCC 2014). How these emergent frameworks, rules, and norms address anthropogenic impacts on ecosystem and natural and working land carbon cycling, feedbacks to climate change, and attribution of emissions to particular sources is a salient question facing practitioners of environmental stewardship and management (e.g., California Air Resources Board 2015). And as considerations of the ecology of carbon and nitrogen cycle science migrate from academic scientific research and enter the world of environmental management and stewardship decision-making, understanding the politics and social-ecological context of carbon and nitrogen cycle science use in practice is a salient question for scholars of environmental governance (Lovbrand and Stripple, 2011, Harris and Symons 2013).

In addition to these these new developments in environmental accounting, we have also witnessed a parallel rise in the interest in assessing and quantifying values produced by ecosystems, generally termed “ecosystem services” (Costanza et al. 1997, Daily et al. 2009). Recently, the ecosystem services framework has also begun to migrate from the realm of scholarly research and development directly into environmental management decision-making (Maler et al. 2008). NGOs, academic scientists, and regulatory agencies have recently developed new guidelines and frameworks for how to consider ecosystem services in decision-making (Olander et al. 2015). And, in 2015, the federal government announced its intention to directly

consider the values of ecosystem services in all federal agency decision-making (Donovan et al. 2015).

Thus, as both greenhouse gas and other sustainability accounting frameworks, as well as the ecosystem services framework, increasingly become a basis for decision-making across scales of governance, their emergence as dominant paradigms within forms of environmental management for sustainability raises profound questions about the relationship between scientific knowledge about anthropogenic alteration of biogeochemical processes and the social, legal and political contexts in which they are being managed. The consequences of what kinds of information are chosen to be included within these emergent institutional contexts, how that information is framed, packaged, contextualized and articulated, and in what institutional contexts it is advanced and disseminated will strongly influence the ultimate success of environmental stewardship in the Anthropocene (Steffen et al. 2015), and form the organizing themes of my dissertation research.

Sustainability science as a unifying approach to research has emerged as a comprehensive framework for understanding the relationships between the generation of knowledge of earth system functioning and its responses to anthropogenic stressors, and actions that seek to mitigate or adapt to these changes, from the individual scale to the regulatory (Jerneck et al. 2011). Recent attention to the conditions under which management in the face of rapid global change is successful has highlighted that the “knowledge to action” pathway is strongly mediated by the institutional networks and narrative framing of environmental problems (McGreavey et al. 2013). The characteristics of the receiving institutional governance context strongly shape how

new information is used within the “knowledge system”, and what forms of new information are most effective at driving actions (Cash et al. 2003). Because it is centered on actionable knowledge, in addition to focusing attention on how scientific information is used and framed, the sustainability science approach also highlights the need for the production of new scientific knowledge that is produced with knowledge of the decision-making contexts in which such information is likely to be used for actions that advance sustainability. Such scientific inquiry is neither basic, nor applied work, but done in recognition of the broader context of the environmental (and social) problems to which it is connected within a knowledge system (Clark 2007).

Theoretical Approach: Social-Ecological Systems

In adopting a sustainability science approach, my dissertation concerns itself with the biogeochemical dimensions of the challenges of sustainability in the Anthropocene: the perturbation of carbon and nitrogen cycles. In order to investigate the pathways, modalities and relationships between knowledge of biogeochemical cycles and the ecosystem services that they provide and emergent institutional and governance frameworks for managing them, this dissertation adopts a social-ecological systems theoretical framework. I briefly introduce this approach below.

The values derived from nature – including carbon sequestration and nitrogen cycling ecosystem services – are traditionally separated into four distinct categories of resources, along axes of ease of excludability of resource users from the resource and whether the consumption of the resource is rival (i.e., the use of the resource by one user diminishes the use of the resource by another user). Resources and values that

have high excludability and are rival in consumption are private goods. Resources that have high excludability but are not rival in consumption are club goods. Common-pool resources are those values that have low-excludability for resource users but high rivalry of consumption. Finally, public goods have low excludability and low rivalry of consumption. Although many ecosystem services are considered common-pool resources, the “resources” that are the focus of this work – regulating and supporting carbon sequestration and nitrogen cycling ecosystem services – have generally been considered public goods (e.g. Muradian and Rival 2012).

However, recent developments in environmental management have sought to develop institutional approaches that transmute these public good ecosystem services into values that are discretized and potentially rival in consumption. For example, in light of the increasing recognition of the finite boundaries and limits to ecosystem functioning, numerous workers have begun to define the act of greenhouse gas emissions as ‘consuming part of a finite atmospheric carbon budget,’ in effect (though perhaps not intent) recasting atmospheric carbon dioxide concentrations as a common pool resource (rival in consumption) rather than a public good (Kanitkar et al. 2010; Edenhofer et al. 2013). Similarly, from programs that explicitly involve commodification of or even payments for ecosystem services to accounting schema that attribute emissions from sources and sinks within ecosystems to particular human actions, carbon sequestration ecosystem services are undergoing a transmutation to common-pool resources.

In her seminal work, *Governing the Commons*, Ostrom (1990) advanced an empirical case-study-derived unifying understanding of what makes institutions that

manage common-pool resources effective, despite the problems of excludability. Ostrom's "design principles" highlight the need for common-pool resource management institutions to set clear rules and boundaries, and, critically, to include provisions for responsiveness to stakeholder- and resource-user input. Her findings, and those of the workers that have followed her approach, have highlighted that some amount of "bottom-up" structure is needed in order for institutions to be adaptive enough to successfully overcome problems of the tragedy of the commons when governing the commons (Ostrom 1990, Cox et al. 2010).

Yet these conclusions are in contrast to the framing of both the ecosystem services framework and carbon governance, which have been, to date, largely top-down in orientation (Backstrand 2004; Ernstson and Sörlin 2013; Stripple and Bulkeley 2013). The current institutional design of our nascent carbon and nitrogen cycle ecosystem service management approaches is thus not one that matches the empirical experience for successful common-pool resource management institutions. One contribution of my dissertation is to pose the question: what makes successful institutions that can manage the challenges and problems of carbon and nitrogen cycling? Specifically, does our empirical understanding of environmental governance institutions and what makes them adaptive and successful still apply to the newly emergent environmental governance institutions that seek to manage ecosystem services, or is a new framework needed to understand these emergent institutions?

Given this central concern, my dissertation assesses institutions constructed around managing carbon and nitrogen cycle ecosystem services as common pool resources. Ostrom's work on is not without its critics, including Young (2002) who

laid out an argument for a more institutionalist, rather than community-focused, approach to assessing earth system governance, and Backstrand et al. (2009), whose empirical assessments of governance institutions focus more on narrative and discourse deployment than on rule- and norm-structures, and Ostrom herself who recognized some of her work's limitations (Ostrom et al. 1999).

In 2009, Ostrom developed comprehensive framework for studying the sustainability of social-ecological systems, which was subsequently updated in 2013 (McGinnis and Ostrom 2013). The social-ecological systems framework is intended to be used for comparative approaches to assessing the sustainability of institutions, and it has been used repeatedly since its initial publication (e.g. Leslie et al. 2015). I adopt it here within the case studies that comprise this dissertation, not in claiming that it must apply to the question of new forms of ecosystem service governance, but in order to begin the process of exploring the knowledge to action pathways that surround carbon and nitrogen cycle management, and in what ways our understanding of existing resource-management institutions and their adaptability can guide

The social-ecological systems framework, originally developed to apply to the management of common-pool resources, but later extended to cover all resource types including public goods, divides the consideration of a given resource within a given social-ecological system context into four compartments: (1) **the resource units**: the resource or value being produced/consumed or used, (2) **the resource system**: the biophysical and ecological processes that produce or generate units and in which units interact with other biophysical components), (3) **the governance system**: the rules governing generation and use of the resource, whether formal laws and regulations or

informal, (4) **actors**: the producers, consumers of the resource, and stakeholders in the use of the resource. These four compartments interact with each other in **action situations** (decision-making contexts) that are shaped by the characteristics of all four of the components simultaneously.

I use this framework to situate each of my six chapters. My coverage is not comprehensive (i.e. not all compartments or pathways and interactions between compartments are addressed for a given resource in a given social-ecological system). Rather, by using this framework my research seeks to inform the understanding of one of these interaction pathways within the context of the broader management of a given resource in a given social-ecological system context.

Organization of Dissertation

At its core, my dissertation asks: What are the conditions under which governance approaches to the management of human alterations of the carbon and nitrogen cycles work best, and what social, technical, and institutional barriers exist to successful adaptive approaches to address the challenges of the Anthropocene?

In order to address these overarching questions, and using the framework for assessing the sustainability of social-ecological systems (Ostrom 2009) to consider the sustainable management of ecosystem carbon and nitrogen cycling, the research in this dissertation is organized thematically into three parts, each comprising two chapters. Part I engages with questions of the dynamics of the “resource system” itself, addressing questions of our understanding of carbon cycle feedbacks to anthropogenic stressors across scales. Part II focuses on relationships between the “resource units”

(in this case, carbon and nitrogen cycle ecosystem services), and both “resources users” and the “governance system”, taking the management of coastal ecosystem carbon sequestration as a social-ecological system case study for these analyses. And finally, through the case of water quality dynamics in San Francisco Bay, Part III explores the relationship between the “resource system”, defined at an ecological rather than an ecosystem service scale, and the “governance system” using the example of state-level water quality management in California. Each of these research themes and its constitutive chapters is introduced below.

Part I: Carbon Cycle Feedbacks

The Fate of Carbon Sinks Under Anthropogenic Global Change

In the process of moving from knowledge of earth system dynamics to rule- and norm-driven actions that seek to measure and regulate human impacts on C and N cycling, ecologists and earth scientists who study the responses of ecosystems to anthropogenic global change have a critical role to play in the continuous process of generating understanding of how human beings are influencing biogeochemical dynamics. While the ability of social-ecological systems to adapt to paradigm-shifting advances in knowledge is the focus of studies of adaptive environmental management and social-ecological resilience (Folke et al. 2002), significant gaps remain in the fundamental understanding of the dynamics and feedbacks of global biogeochemical cycles to anthropogenic perturbation that are highly relevant to our current governance contexts (e.g. Gardenas et al. 2011).

In Part I, I concern myself with the feedbacks and fate of carbon cycling within ecosystems that are undergoing rapid anthropogenic global change. Using both observational and experimental approaches, I seek to assess how much carbon is exported, lost or sequestered from ecosystems whose earth system functioning is being altered by climate change and other anthropogenic stressors. The goal of both chapters is to provide insight into how the carbon cycle of a future world is likely to function. Understanding such feedbacks – whether climate change may lead to greater sequestration of carbon, greater loss of carbon, or simply alter the rate of carbon cycling – is critical both to parameterizing earth system models, and to tailoring climate change mitigation policies that seek to account for anthropogenic impacts on net carbon emissions and sinks from ecosystems.

Chapter 1 explores these questions in the rapidly changing Chukchi Sea shelf ecosystem. The Chukchi Sea is located in the Pacific Arctic and has experienced rapid changes in the dynamics of sea ice, with sea ice retreating earlier and farther north over the summers of the most recent two decades (Frey et al. 2015). The Chukchi Sea is also one of the most productive coastal ecosystems on the planet, and primary production has been increasing as sea-ice has been retreating (Arrigo and van Dijken 2011). This phytoplankton production drives a productive benthic food-web that is the food source for migrating gray whales, walrus, king eider ducks and other species of concern (Grebmeier et al. 2006). The Chukchi Sea also represents a strong atmospheric carbon sink, but it remains unclear if this sink expanding in its potential potency as sea ice retreats. Using data obtained from two oceanographic cruises to the region, I assess what fraction of the carbon fixed by phytoplankton in this shallow

shelf sea is exported out of the system, what fraction of this carbon is likely to remain out of contact with the atmosphere (and thus be sequestered), and what drives variability in the fate of carbon from this locally and globally critical ecosystem.

Chapter 2 assesses the emissions of soil CO₂ from California grassland soils that were exposed to a punctuated single disturbance event: an experimental wildfire, in combination with decade-scale duration global change treatments of elevated temperature, CO₂, precipitation and increased nitrogen deposition. While previous studies have identified that soil CO₂ efflux rates increase with short-duration warming (Melillo et al. 2002), measuring the effects of long-duration, decade scale global change in combination with disturbances such as fire is critical to improving our ability to monitor changes in C stocks and soil C releases, and to understanding how such feedbacks to climate change might adjust over time scales longer than short-duration experiments. The results of this work can both help to inform the development of approaches to land-sector GHG accounting for climate mitigation policies, and help to improve the characterization of climate-carbon cycle feedbacks and constraining earth system models that help predict future ecosystem functioning (Reichstein et al. 2008).

Part II: Coastal Ecosystem Service Management

Examining Pathways of Action within New Governance Institutions: The Uptake of the Ecosystem Services Framework in the Coastal Zone

Understanding the relationship between carbon cycle science and climate mitigation actions is a fundamental component of sustainability science, and the

framing and understanding of carbon cycle components in environmental governance is a critical determinant of the trajectory of future climate governance (Lovbrand et al. 2009).

Part II of my dissertation approaches this relationship through two studies of the treatment of blue carbon in contemporary governance practice. Blue carbon refers to the sequestration and storage of atmospheric carbon in tidal flats, sea grass meadows, coastal mangrove forests, salt marshes and tidal wetlands, and kelp forests (McLeod et al. 2011). Such ecosystems globally sequester 100s of millions of tons of CO₂ annually, making them among the most powerful carbon sinks per unit area on the planet (Chmura et al. 2003). These coastal ecosystems are strong carbon sinks because of low rates of remineralization associated with low oxygen environments in wetland soils, and because of the high capacity of some blue carbon ecosystems such as sea grasses to trap allochthonous organic carbon (Greiner et al. 2013).

In Chapter 3, I explore how blue carbon ecosystem services are currently being used in local-scale coastal conservation practice by coastal conservation organizations. In particular, I assess what barriers exist to organizations adopting, using, or acknowledging the ecosystem services framework within their work. In light of rapid institutional construction and interest, understanding how local stakeholders in coastal ecosystem services engage with these concepts will be critical to determining the ultimate trajectory of the use of ecosystem services in decision-making, currently envisioned in federal agency processes (Donovan et al. 2015). Through semi-structured interviews with nearly three dozen leaders of coastal conservation organizations, I present a typology of the barriers to and conditions under which the

ecosystem services framework is penetrating local-scale, place-based conservation practice.

Chapter 4 addresses very similar questions in the context of formal regulatory implementation, in this case of the National Environmental Policy Act. Several studies have recently elucidated pathways through which ecosystem services, and in particular those services related to carbon cycling, can be incorporated into a wide-range of existing statutory contexts (Ruhl 2010; Sutton-Grier et al. 2014). In particular, Sutton-Grier et al. (2014) highlighted that carbon sequestration could be directly “read into” existing federal statutory authorities, including the Coastal Zone Management Act and National Environmental Policy Act. Here, we assess the *status quo* treatment of blue carbon and other ecosystem services within the NEPA process, focusing particularly on environmental impact statements produced for federal actions taking place in the coastal zone. Our analysis seeks to identify the current conceptualization of carbon cycle ecosystem services in order to focus the attention of both agencies and scholars on the modalities and practices of adaptive management in the context of rapid global change.

PART III: Estuarine Water Quality and Management

Knowledge to Action and Back Again: Exploring Estuarine Water Quality in a Policy-Relevant Contested Science Space

While Parts I and II are focused on both the science and governance of climate change mitigation, Part III adopts a case study approach focused on conducting policy-relevant experimental and observational research on the nitrogen, rather than the

carbon cycle, to address a single environmental puzzle: the high anthropogenic nitrogen loading, but low productivity of the San Francisco Bay Estuary, and whether or not ammonium from wastewater effluent is the cause of low productivity. The San Francisco Bay estuary, which drains California's Central Valley, is the largest estuary on the Pacific coast of North America, and is an ecologically, commercially, and culturally important social-ecological system. Since the 1980s, the northern portion of this estuary has experienced significant declines in phytoplankton productivity, which have cascaded up the food-web leading to the phenomenon of pelagic organism decline (POD) (Sommer et al. 2007). Several proposed drivers of the phytoplankton crash linked to POD have been advanced in the literature, including light limitation (Jassby and Cloern 2012), grazing by an invasive clam species (Lopez et al. 2006), and high anthropogenic N loading as ammonium (NH_4^+) from wastewater effluent (Glibert 2010, Parker et al. 2012, Dugdale et al. 2007).

In Chapter 5, I present the results of an experimental study of phytoplankton production, inorganic nitrogen uptake rates, and changes in phytoplankton community composition in response to experimental additions of three different nutrient amendments: added ammonium (NH_4^+), added nitrate (NO_3^-) and added filtered wastewater effluent, at two different irradiance levels. The experiment was designed to test whether phytoplankton growth or community composition varied as a function of the form of available N. We were particularly interested in whether NH_4^+ from wastewater effluent in the Sacramento River suppressed NO_3^- uptake rates by phytoplankton, suppressed total production, or influenced the composition of the phytoplankton community. These questions are salient to decision-makers faced with

the management of nutrient inputs and their effects on water quality in the Lower Sacramento River and San Francisco Bay. In addition to being prepared for publication, the results of this experiment are being presented directly to the Central Valley Regional Water Board, which participates in California's implementation of federal Clean Water Act and grants National Pollution Discharge Elimination System (NPDES) permits.

In Chapter 6, I describe insights derived from a year of monthly water quality cruises in San Francisco Bay. Using high-frequency spatial sampling techniques to measure surface ammonium and nitrate concentrations, and surface phytoplankton physiological state, we are able to derive insights into key ecological questions about phytoplankton responses to environmental controls in this highly-impacted estuary. Here, with an eye to the pathway through which such information can be used within the existing institutional context of federal, state, and municipal water quality regulations, we present results on dynamics and drivers of poor phytoplankton physiological conditions in Suisun Bay. Our results indicate that high concentrations of NH_4^+ from wastewater are not the driver of this phenomenon.

Global Change Ecology and Environmental Governance

Through the studies of these six chapters, this dissertation is situated at the nexus of the ecological understanding of the dynamics of anthropogenic global change and the understanding of emergent institutions of environmental governance that are actively being constructed to address the biogeochemical challenges of earth system governance in the Anthropocene.

Chapter 1

Mass balance estimates of carbon export in different water masses of the Chukchi Sea shelf¹

ABSTRACT

In this chapter, we construct mass-balance based estimates of carbon (C) export fractions from the water column across the Chukchi Sea shelf. Export is calculated as the difference between phytoplankton drawdown of dissolved inorganic C (DIC) and the accumulation of autochthonous particulate and dissolved organic C in the water column. Organic carbon (C_{org}) exports of >50% of DIC drawdown are ubiquitous across the shelf, even during, or shortly after, phytoplankton blooms, suggesting widespread and strong pelagic-benthic coupling. Export fractions on the shelf were generally greater in the less-productive Alaska Coastal Water than in the more productive Bering Shelf-Anadyr Water. Additionally, export fractions were greater in 2011 than in 2010, highlighting the significant spatial and inter-annual variability of the fate of C_{org} in this ecologically and biogeochemically important, and rapidly changing, ecosystem.

INTRODUCTION

The response of primary production to changes in sea ice cover is of central interest to the Arctic community (Schofield et al. 2010). Characterizing this response

¹ This chapter was written with input and contributions from Kate Lowry, Zachary Brown, Matt Mills, Gert van Dijken, Bob Pickart, Lee Cooper, Karen Frey, Ron Benner, Cedric Fichot, Nicholas Bates and Kevin Arrigo. A version of this study is currently in press in *Deep Sea Research II: Special Topics in Oceanography*.

allows us to understand how climate change will influence Arctic marine ecosystems, as well as how the cycling of carbon (C) and nitrogen (N) in these systems will affect global C and N budgets. As an example, the Chukchi Sea shelf in the Pacific Arctic exhibited a 48% increase in net primary production (NPP) between 1998 and 2009 (Arrigo and van Dijken, 2011) (though see Yun et al. 2014 for evidence of lower primary production on the shelf in 2009). It has been suggested that more exposed open water, staying ice-free for longer, with greater biological productivity in the surface layer, leads to a greater air-sea flux of carbon dioxide (CO₂) (Bates and Mathis, 2009), and that any extra drawdown enhances the CO₂ sink, acting as a negative feedback on climate change.

Ultimately, however, any changes in net flux of CO₂ are only a significant feedback to climate change if the associated C removed from the atmosphere enters and remains in the deep ocean, where it is no longer in equilibrium exchange with the atmosphere, or is buried in the sediments. Even if primary production increases substantially on the shelf, if the resulting organic C is remineralized back to CO₂ in shallow waters and re-equilibrates with the atmosphere, the net effect on the C cycle is only an increase in the rate of ocean-atmosphere C cycling, but not an increase in the net oceanic storage of C. In the deep ocean, a fraction of C_{org} fixed by photosynthesis sinks below the mixed layer and is exported to depth, sequestering it from exchange with the atmosphere. The fraction of fixed C_{org} that is exported from the surface ocean through this ‘biological pump’ is a critical global determinant of the fate of anthropogenic CO₂ in the atmosphere (Ducklow et al. 2001).

On shallow (<150m) continental shelves where the entire water column mixes in the winter due to sea ice formation, C_{org} export is not sequestered below the mixed layer. In shallow shelf systems, fixed C_{org} is either remineralized and exchanged with the atmosphere, accumulates in benthic sediments, or is transported off of the shelf. Many such continental shelves exhibit the capacity to “pump” atmospheric C to the deep ocean by advecting dense coastal C-rich water off the continental shelf, through a process known as the “continental shelf pump” (Tsunogai et al. 1999). There is evidence for an active continental shelf pump in the western Arctic Ocean, including the Chukchi Sea and East Siberian Sea shelves (Bates 2006; Anderson et al. 2010). Results from the Shelf-Basin Interactions (SBI) project show significant, but seasonally variable, rates of C export off the northeastern Chukchi Sea shelf (Moran et al. 2005; Lalande et al. 2007b; Lepore et al. 2007). Understanding the spatial variability of rates of C_{org} export from the vast productive Chukchi Sea shelf is critical to understanding the response of the C cycle to reductions in sea ice duration and extent in this region.

The Chukchi Sea is a shallow continental shelf sea located in the western Arctic Ocean, bounded on the southwest by the Chukotka Peninsula and on the southeast by northwestern Alaska. It has an average shelf depth of ~50 m (Hameedi, 1978), and there is a pronounced shelf-break that runs northwest to southeast, separating the Chukchi Sea shelf from the Canada Basin (Weingartner et al. 2005). Pacific water flows onto the shelf from the Bering Strait (Figure 1-1), originating from three distinct sources. The first is the nutrient-rich water from the Gulf of Anadyr and the second is water from the central Bering Sea shelf. These two water masses flow through the

western portion of the Bering Strait (Hansell et al. 1993; Cooper et al. 1997; Weingartner et al. 2005), and mix in the southern Chukchi Sea to form Bering Sea Water (BSW, Coachman et al. 1975). The third Pacific water source is Alaskan

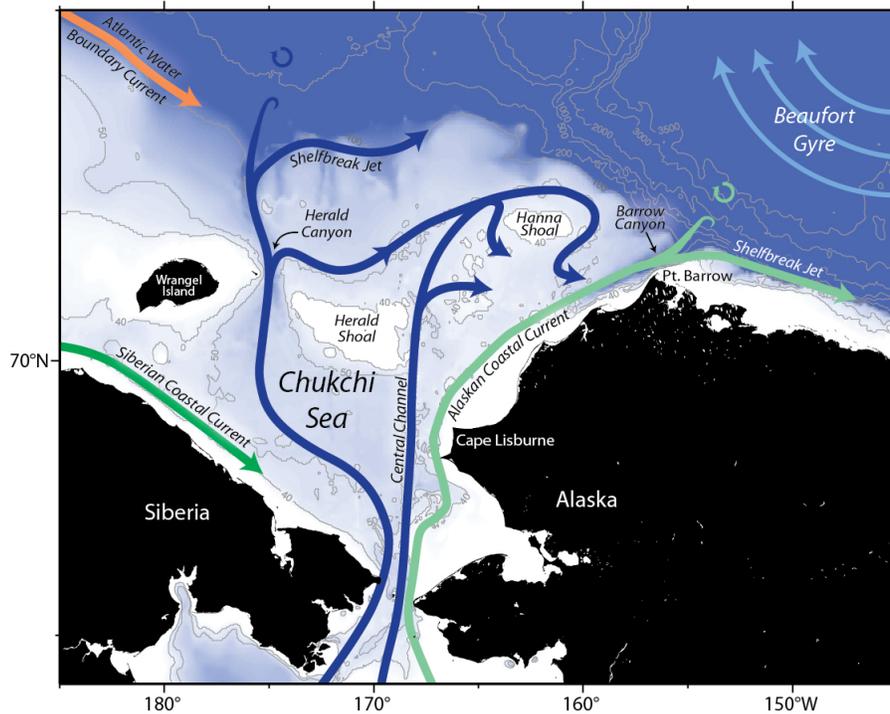


Figure 1-1. Schematic circulation of the Chukchi Sea shelf region (after Brugler et al. 2014), including place names. Water enters the shelf through the Bering Strait. The Bering Sea Water (BSW) flow pathways, which progress across the shelf around Herald and Hanna Shoals, are shown in dark blue. The Alaska Coastal Current flow path, which follows the coast, is shown in light green.

Coastal Water (ACW), which is warmer and fresher than BSW and lower in nutrients. It is advected northward by the Alaskan Coastal Current (ACC) and flows through the eastern portion of the Bering Strait predominantly in summer (Paquette and Bourque, 1974; Weingartner et al. 2005)

It is believed that the BSW and ACW progress northward in the Chukchi Sea predominantly along three main pathways. One of these pathways is the ACC (Figure

1, shown in light green), which comprises ACW, and two of these pathways comprise BSW: a central branch and a western branch. In the central branch, BSW follows a pathway through the Central Channel (between Herald and Hanna shoals) and in the western branch, BSW flows through Hope Valley into Herald Canyon (Figure 1-1, shown in dark blue). As described in Pickart et al. (2016 and shown in Figure 1-1, some of the water in the western branch is diverted eastward north of Herald Shoal and joins the Central Channel branch. As this water encounters Hanna Shoal it then bifurcates and flows around each side of the shoal. In addition, some of the water in the Central Channel pathway appears to leak through gaps in the ridge between Hanna and Herald shoals. Ultimately much of the Pacific water entering through Bering Strait drains through Barrow Canyon, particularly during the summer months (Itoh et al. 2015; Gong and Pickart, 2015; Pickart et al. 2016). Notably, the middle of Barrow Canyon exhibits high benthic biomass (Grebmeier, 2012). This is the location where high-nutrient Pacific winter water exits the shelf in late-summer (Itoh et al. 2015) and also where wind-driven upwelling can bring this water back into the canyon from the Canada Basin (Mountain et al. 1976; Aagaard and Roach, 1990; Pickart et al. 2013). An eastward flowing shelf-break jet flows along the northern edge of the Chukchi Sea (Pickart et al. 2005; Corlett and Pickart, submitted), which can spawn eddies that transport organic carbon off the shelf (Mathis et al. 2007a).

Sea ice retreats from south to north across the shelf, beginning in May each year. From 1979 to 1998, sea ice remained over the northern portion of the Chukchi shelf in summer. Since then, however, the ice has regularly been retreating past the shelf-break each summer, and, since 2007, it has retreated fully or nearly-fully off the shelf each

year. In the Chukchi Sea, the mean open water area has increased by 1000 km² per year over the last decade, and the duration of the open water season has also significantly increased (Brown and Arrigo, 2012; Frey et al. 2015). There is some evidence that these patterns of retreat are due to changes in annual mean heat flux through the Bering Strait (Shimada et al. 2006; Woodgate et al. 2012).

Sea ice retreat strongly determines the variability of phytoplankton production on the Chukchi shelf (Grebmeier et al. 2010; Wang et al. 2013). Prior to the discovery of under-ice phytoplankton blooms (Arrigo et al. 2012), it was believed that phytoplankton production in the Chukchi began each year in late-May as the ice retreats, peaking around 10 June (Arrigo and van Dijken, 2011). Zooplankton grazing rates of these blooms are generally considered to be low (Sherr et al. 2009), allowing a large portion of the production to settle to the benthos without being grazed (Grebmeier, 2012).

Because of relatively low grazing rates in cold water at the onset of the blooms, the Chukchi shelf is characterized by significant pelagic-benthic coupling, as early season sea ice algal production and spring phytoplankton blooms support significant benthic production (Grebmeier et al. 1988; Grebmeier et al. 2006). The C_{org} from the blooms that reaches the sea floor fuels one of the most productive soft-bottom benthic ecosystems on the planet; levels of macroinfaunal biomass of 60 g C m⁻² are found in southern portions of the Chukchi shelf (Grebmeier, 1993). Benthic production represents a significant food source for the Pacific walrus (*Odobenus rosmarus divergens*) (Fay, 1982) and summer-feeding gray whales (*Eschrichtius robustus*) (Clarke et al. 1989; Moore et al. 2003). As such, changes in sea ice may have

significant implications for both pelagic and benthic ecosystems (Grebmeier et al. 2006; Wassmann and Reigstad, 2011; Grebmeier, 2012). Previous studies have suggested that there is no long-term accumulation of C_{org} on the shelf (Mathis et al. 2007b), and that a significant proportion of fixed C is likely transported off the shelf and into the Canada Basin after remineralization (Bates et al. 2005b).

The organic C produced by marine phytoplankton blooms also fuels benthic denitrification, the microbial enzyme-mediated conversion of fixed N to gaseous N_2 (Devol et al. 1997; Chang and Devol, 2009; Granger et al. 2011). Owing to the fact that the Pacific water traverses the shallow Chukchi shelf before being advected into the basin and ultimately exiting the Arctic through Fram Strait and the Canadian Arctic Archipelago, the rates of denitrification on the shelf play a globally significant role in N removal, stimulating intensive nitrogen fixation in the North Atlantic (Yamamoto-Kawai et al. 2006).

Photosynthesis by phytoplankton blooms on marginal continental shelves in the Arctic can substantially lower the water column partial pressure of carbon dioxide (pCO_2), driving a net air to sea flux of CO_2 during the seasonally ice-free season (Arrigo et al. 2010). Continental shelves in the Arctic are generally thought to be net “sinks” for atmospheric CO_2 (Chen and Borges, 2009), and the Chukchi Sea shelf has been demonstrated to be a strong and increasing CO_2 sink (Bates et al. 2006), though the strength of the sink itself may be decreasing (Cai et al. 2010). Satellite-derived estimates of total annual NPP in the Arctic Ocean are on the order of 500 Tg C yr^{-1} (Arrigo and van Dijken, 2011), which is thought to represent a significant net metabolic sink of atmospheric CO_2 , on the order of $100\text{-}200 \text{ Tg C yr}^{-1}$ (Arrigo et al.

2010). This represents 5-14% of the global C cycle balance (Bates and Mathis, 2009). Thus, in addition to fueling productive benthic ecosystems on the shelf itself, the fate of production in these shallow waters is also a significant potential determinant of the global N and C cycles.

In this study, we construct mass-balance based estimates of C_{org} export across the Chukchi Sea shelf, including central and southern portions of the shelf, using data collected during the NASA-sponsored program Impacts of Climate on the Ecosystems and Chemistry of the Arctic Pacific Environment (ICESCAPE). As part of ICESCAPE, oceanographic cruises to the Chukchi Sea were carried out in summer 2010 and summer 2011. We assume that the nutrient-rich winter water on the shelf during each of the surveys represents pre-bloom conditions and, based on this assumption, we compute the C_{org} export of the nutrient-poor summer waters. Our objective is to understand how the amount of C_{org} exported from the water column varies across this ecologically significant continental shelf and to compare our estimates with those made previously in the region. Such understanding is critical for assessing the implications of changes in sea ice, water temperature, and increases in NPP for this highly productive ecosystem.

METHODS

Study Location and Sampling

We assessed export of fixed C from the water column on the shallow Chukchi shelf during two consecutive summer field campaigns (15 June to 21 July 2010 and 25 June to 29 July 2011), conducted as part of the ICESCAPE project. Numerous

transects were occupied across the shelf during each survey (Figure 1-2). A total of 140 stations were occupied in 2010 and 173 stations in 2011. Seawater samples were taken from discrete depths throughout the water column at each station. Typical sampling depths included 2, 10, 25, and 50 m, the depth of maximum chlorophyll a (Chl a) concentration, and approximately 2 m above the seafloor. At stations on the continental slope, additional samples were typically taken at depths of 100, 150, and 200 m.

At each station, seawater was collected from 30 L Niskin bottles attached to a rosette that included a SBE 911+ conductivity-temperature-depth (CTD) sensor. The water samples collected from each depth at each station were analyzed for Chl a concentration, dissolved inorganic C (DIC) concentration, nitrate+nitrite (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), and dissolved silicate ($\text{Si}(\text{OH})_4$) concentrations. From selected stations, samples were also analyzed for dissolved organic C (DOC) concentration, water oxygen stable isotope ratios ($\delta^{18}\text{O}$) and particulate organic C (POC) concentration. Salinity measurements were made at a minimum of two discrete depths at each station to calibrate the CTD-mounted conductivity sensor.

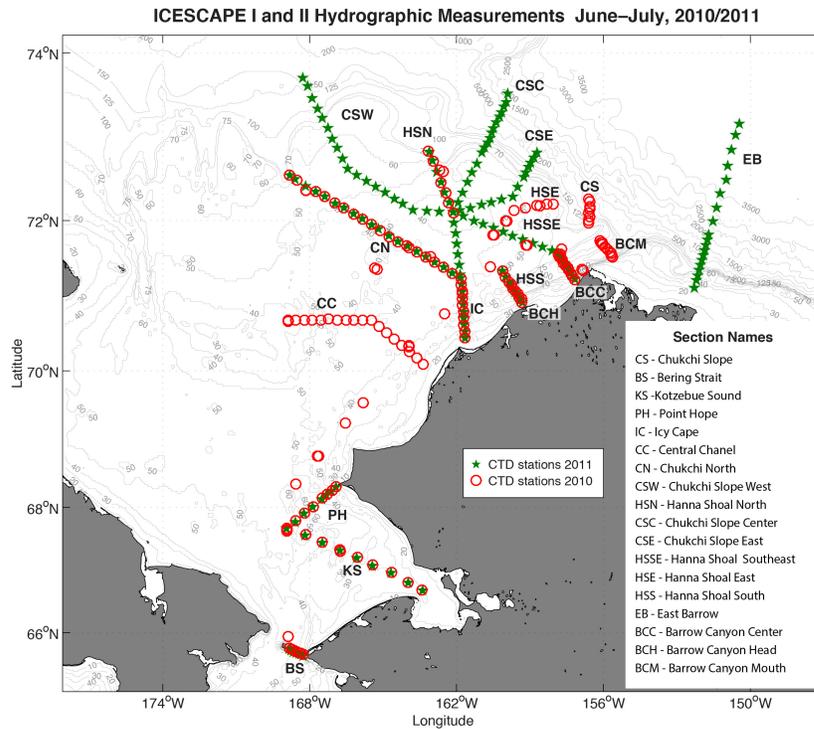


Figure 1-2. Sampling transects of the ICESCAPE project are shown for 2010 and 2011.

Sample Analyses

Samples for fluorometric analysis of Chl a were filtered onto 25 mm Whatman glass fiber filters (GF/F, nominal pore size 0.7 μm), placed in 5 mL of 90% acetone, and extracted in the dark at 3°C for 24 hrs. Chl a was then measured fluorometrically (Holm-Hansen et al. 1965) using a Turner Fluorometer 10-AU (Turner Designs, Inc.).

DIC samples were collected in 300 mL borosilicate bottles, poisoned with 100 μL mercuric chloride (HgCl_2), and stored in the dark until analysis using a gas extraction/coulometric detection system. Samples collected in 2010 were analyzed at the University of Alaska Fairbanks, and samples collected in 2011 were analyzed shipboard and at Bermuda Institute for Ocean Sciences.

POC was analyzed by filtering seawater onto pre-combusted 25 mm Whatman GF/F. The filters were fumed with hydrochloric acid (HCl), dried at 60°C, and transferred to tin capsules (Costech Analytical Technologies, Inc.) for analysis on an Elementar Vario EL Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) at the University of California, Davis.

Seawater samples for DOC analysis were gravity-filtered directly from Niskin bottles through pre-combusted Whatman GF/F (0.7 µm pore size), collected into pre-cleaned (10% hydrochloric acid solution) 60 mL Nalgene High-Density-PolyEthylene (HDPE) bottles, and stored frozen immediately until analysis. DOC analysis was conducted within three months of collection by high-temperature combustion using a Shimadzu total organic carbon TOC-V analyzer equipped with an autosampler (Benner and Strom, 1993, Shen et al. 2012) at the University of South Carolina. Blanks were negligible and the coefficient of variation between injections of a given sample was typically 0.6%. Accuracy and consistency of measured DOC concentrations was checked by analyzing a deep seawater reference standard (University of Miami) every sixth sample.

$\delta^{18}\text{O}$ samples were returned to the Chesapeake Biological Laboratory of the University of Maryland Center for Environmental Science in sealed vials with precautions undertaken to prevent evaporation (and isotopic fractionation). Water samples were analyzed by equilibration with CO_2 using a Thermo Fisher Gas Bench II peripheral linked to a continuous flow Delta V Plus isotope ratio mass spectrometer. Analytical precision was better than $\pm 0.1\text{‰}$ and was assessed by analysis of in-house water standards during sample analysis and calibration to international water isotope

standards (V-SMOW, SLAP, GISP). Data were normalized as per recommendations of Paul et al. (2007).

Prior to the calculations of C_{org} export, all concentration values were salinity-normalized to a winter water salinity value of 33.1, following Bates et al. (2009), to account for dilution by sea ice melt at the surface.

Calculating Particulate Organic Carbon Export

C_{org} export from the summer waters of the shelf was estimated for each station using a mass balance approach, defined as the difference between the estimated drawdown of DIC and the accumulation of POC+DOC in the summer water from pre-growth winter water values. Thus, the C exported out of the water column is calculated as the amount of C_{org} that was fixed by phytoplankton, but is not present in the observed particulate or dissolved organic form in the water column. This mass-balance approach provides an estimate of an instantaneous amount of particulate organic C exported rather than a rate of export in units of time. We express this mass-balance derived C_{org} export estimate as an absolute mass (mmol C m^{-2}) and as a % export fraction, relative to the amount of inorganic C drawdown by photosynthesis. This approach also accounts for remineralization within the water column, as DIC drawdown is calculated using measured DIC concentrations that would include any remineralized C (DIC drawdown is thus equal to net community production (NCP)). Because we are interested in shelf-stations, we limited our analysis to stations with depths <150 m.

The equations for the amount of C_{org} exported from the water column, and the %C exported, at a given station, in units of mmol C m^{-2} are:

$$C_{\text{exp}} = \Delta\text{DIC}_i - (\Delta\text{POC}_i + \Delta\text{DOC}_i) \quad (1.1)$$

and

$$\% C_{\text{exp}} = (\Delta\text{DIC}_i - (\Delta\text{POC}_i + \Delta\text{DOC}_i)) / \Delta\text{DIC}_i \quad (1.2)$$

where ΔDIC_i is the depth-integrated DIC deficit, ΔPOC_i is the depth-integrated POC (the delta indicates an accumulation from a baseline of close to 0), and ΔDOC_i is the depth integrated accumulation of DOC.

ΔDIC_i is defined as

$$\Delta\text{DIC}_i = \text{DIC}_{\text{ww}} * z - (\sum((\text{DIC}_{mz} + \text{DIC}_{mz+1})/2) * \Delta z) \quad (2)$$

where DIC_{ww} is the estimated pre-bloom, winter water DIC concentration in mmol C m^{-3} , DIC_{mz} is the measured, salinity-normalized DIC concentration of summer water at sampling depth z , DIC_{mz+1} is the measured salinity-normalized DIC concentration at the adjacent sampling depth, and Δz is the vertical difference (m) between sampling depths. It is assumed that, in pre-growth conditions, winter water DIC concentrations are generally uniform across the Chukchi Sea shelf. As such, our estimates of DIC_{ww} were taken as representative across all shelf stations, even if no remaining winter water was present at the time of sampling.

ΔPOC_i is defined as

$$\Delta\text{POC}_i = \sum((\text{POC}_{mz} + \text{POC}_{mz+1})/2) * \Delta z \quad (3)$$

where POC_{mz} is the measured concentration of summer water POC in mmol C m^{-3} at depth z and POC_{mz+1} is the POC concentration of at the adjacent depth. POC is listed assumed to be close to zero prior to the onset of phytoplankton production.

Finally, ΔDOC_i is defined as

$$\Delta\text{DOC}_i = \sum(\text{DOC}_{mz} + \text{DOC}_{mz+1})/2 * \Delta z - \text{DOC}_{\text{ww}} * z \quad (4)$$

where DOC_{mz} is the measured summer water DOC concentration in $\mu\text{mol L}^{-1}$ at depth z and DOC_{ww} is the mixing-model derived winter water concentration of DOC for that year.

ΔDIC_i , the water-column DIC deficit, represents a per-meter squared estimate of the difference between the amount of DIC that was in the water-column prior to phytoplankton growth, and the amount remaining in the water column at the time the sample was taken. Winter water DIC concentrations were estimated by first identifying all sampling depths with temperatures of $\leq -1.6^\circ\text{C}$ (Pickart et al. 2016) and NO_3^- concentrations $\geq 10 \mu\text{mol L}^{-1}$ (to avoid including surface water), and then by calculating the mean salinity-normalized concentration of DIC for those locations in that year. Winter water defined as such was present at 51 individual depths at stations in water <150 m deep in 2010 and at 102 individual depths at stations in water <150 m deep in 2011. Our winter water samples had a mean salinity of 33.0 ± 0.2 in 2010 and 32.8 ± 0.3 in 2011 corresponding closely the value of 33.1, which is salinity of the upper halocline of the Arctic Ocean. In 2010, the mean winter water DIC concentration was $2229 \pm 56 \mu\text{mol C L}^{-1}$, with a maximum concentration of $2301 \mu\text{mol C L}^{-1}$. In 2011, the mean winter water DIC concentration was $2272 \pm 28 \mu\text{mol C L}^{-1}$, with a maximum concentration of $2356 \mu\text{mol C L}^{-1}$. These estimated winter water values are slightly lower than, but generally consistent with, shelf-wide winter water measurements made during the pre-bloom spring portion of the SBI project (Bates et al. 2005a). Because DIC_{ww} is an average of winter water values taken from stations for each year of the cruise, for a given station and depth, it is possible for the measured DIC in the summer water to exceed the calculated winter water DIC. If $\text{DIC}_{mz} >$

DIC_{ww}, there was assumed to be no DIC deficit (relative to winter water) at that station and depth.

For the ΔPOC_i calculation, we assumed that there was no remnant POC in the water column prior to spring phytoplankton growth, since organic matter produced in previous years would have been either remineralized, transported off of the shelf, or deposited to the bottom.

DOC concentrations in Arctic waters are strongly influenced by the input of DOC from riverine sources (Dittmar and Kattner, 2003), and the Chukchi Sea shelf is no exception, with the majority of riverine DOC entering the Chukchi Sea shelf originating from the Yukon River and passing through the Bering Strait (Anderson 2002). Allochthonous riverine DOC is thought to be relatively resistant to degradation and is present year-round in waters on the Chukchi Sea shelf (Mathis et al. 2005; Cooper et al. 2005). Thus, accumulation of autochthonous DOC derived from phytoplankton drawdown of inorganic C, which is the DOC accumulation that we wish to include in our mass balance calculation, must be calculated relative to background concentrations that are present year round, and these background concentrations vary with the mix of source waters across the shelf.

In particular, on the Chukchi Sea shelf, DOC concentrations are strongly correlated with salinity, reflecting a conservative mixing of water sources. Thus, the amount of DOC at a given location on the shelf is a function of the amount of freshwater that is derived from meteoric water in the water column relative to the amounts of Atlantic basin water and sea ice melt water (Cooper et al. 2005). To assess the background concentrations of DOC, we first used a three-component mixing

model approach described by Cooper et al. (2005) and Mathis et al. (2007b) to determine the fraction of runoff water present at each station.

Measurements of salinity and oxygen stable isotopes in water are used to determine the relative fractions of the three different water components (sea ice melt, Atlantic basin water and runoff water of meteoric origin) at each station and depth on the Chukchi Sea shelf. These fractions were estimated by solving three coupled equations:

$$f_{\text{sim}} + f_{\text{runoff}} + f_{\text{Atlantic}} = 1 \quad (5.1)$$

$$f_{\text{sim}} * \delta^{18}\text{O}_{\text{sim}} + f_{\text{runoff}} * \delta^{18}\text{O}_{\text{runoff}} + f_{\text{Atlantic}} * \delta^{18}\text{O}_{\text{Atlantic}} = \delta^{18}\text{O}_{\text{observed}} \quad (5.2)$$

$$f_{\text{sim}} * \text{Salinity}_{\text{sim}} + f_{\text{runoff}} * \text{Salinity}_{\text{runoff}} + f_{\text{Atlantic}} * \text{Salinity}_{\text{Atlantic}} = \text{Salinity}_{\text{observed}} \quad (5.3)$$

where f is the fraction of each component, “sim” denotes water from sea ice melt, “runoff” denotes freshwater from meteoric water, and “Atlantic” denotes the core Atlantic water in the Arctic Ocean basin. We assume that Atlantic water in the Arctic Ocean basin has a salinity of 34.8 and a $\delta^{18}\text{O}$ value of +0.3‰ (Ekwurzel et al. 2001). Based upon measurements of sea ice during ICESCAPE cruises, we set the salinity of sea ice to 4, with a $\delta^{18}\text{O}$ value of -1‰ (Logvinova et al. 2015). Because freshwater on the Chukchi Sea shelf comes from meteoric water transported north from Bering Strait (Weingartner et al. 2005), we chose a runoff end-member of $\delta^{18}\text{O}$ of -21.35‰, which corresponds to the most up-to-date data for waters collected solely within Bering Strait (Cooper et al. 2006, and unpublished data). The salinity for the freshwater-fraction is set to 0.

Using measured salinity and $\delta^{18}\text{O}$ data for 2010 and 2011 and equations 5.1, 5.2, and 5.3, we estimated the fractions of melted sea ice, Atlantic water and

freshwater derived from meteoric water (runoff) for all samples where isotopic measurements were made (n=513 in 2010; n=640 in 2011).

We calculated DOC_{ww} for use in Equation 4 from $\delta^{18}\text{O}$ -derived data using two approaches. In the first approach, we used the relationship between runoff fraction and DOC to calculate a DOC_{ww} value for each individual station, following Mathis et al. (2007b). In this manner, for a given station and depth, DOC_{ww} is equal to the background DOC concentration for the given mix of sea-ice melt, runoff, and Atlantic water. ΔDOC_i is then measured as the accumulation above that background value.

In the second approach, average runoff fractions were calculated for stations and depths with winter water (defined as described above). Using the relationship between runoff fraction and DOC concentration (shown in Figure 1-3), we then calculated DOC_{ww} from the average runoff fraction for all winter water stations.

Consistent with previously published data from the Chukchi Sea, autochthonous DOC accumulation represented only a small fraction of NCP (Mathis et al. 2007b). In 2010, using the first approach, DOC accumulation represented an average of 5% of DIC drawdown, with a maximum increase of $11 \mu\text{mol L}^{-1}$ DOC from autochthonous production. Using the second approach, DOC accumulation was an average of 7% of DIC drawdown. Likewise, in 2011, these fractions were 12% and 7.5%, respectively, with a maximum increase of $22 \mu\text{mol L}^{-1}$ DOC from autochthonous production in surface waters at one station. Previously published estimates for DOC accumulation as a fraction of NCP for this region were ~10%, with increases of up to $\sim 14 \mu\text{mol L}^{-1}$ (Mathis et al. 2007b).

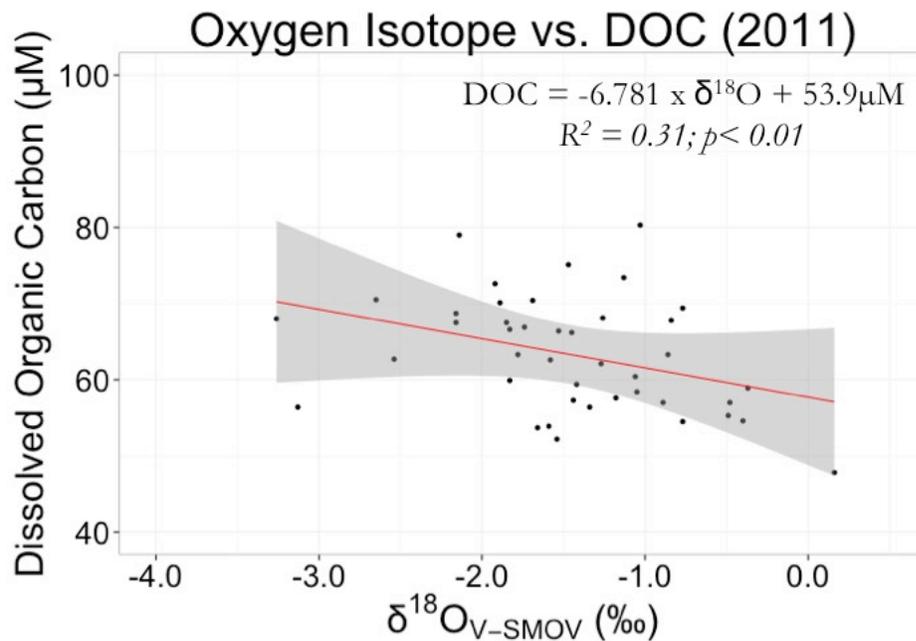
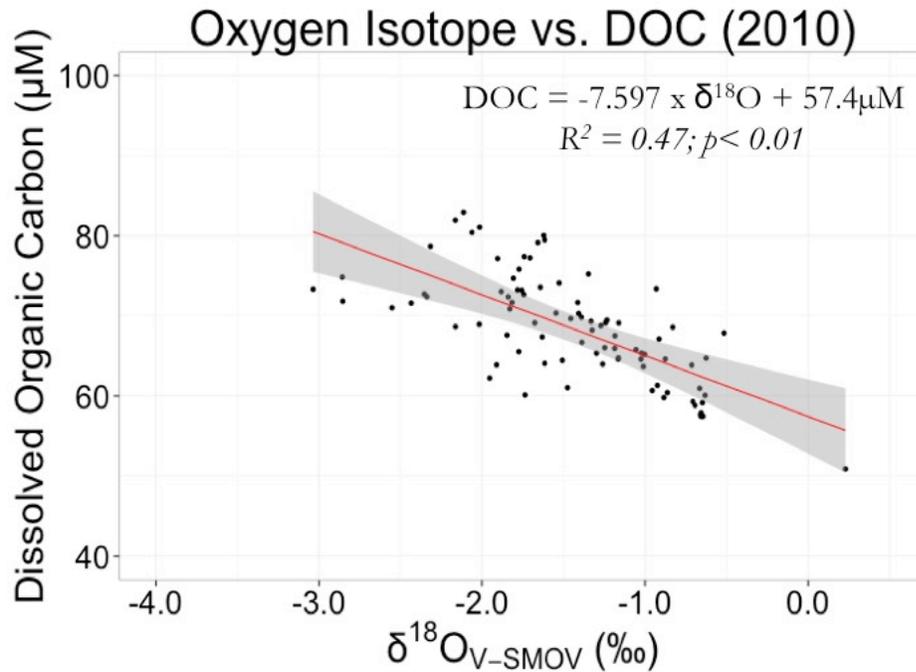


Figure 1-3. Regression for 2010 and 2011 of (A) seawater $\delta^{18}O$ versus DOC and (B) three-component mixing model-derived runoff fractions versus DOC. The best fit linear model is shown in red, with a 99% confidence interval for the model's position shown in gray. The equation and R^2 for each model is given and both models are significant at the $p < 0.01$ level. Background DOC concentrations on shelf waters follow a mixing line of Atlantic origin water, sea ice melt, and meteoric water.

In order to estimate DOC accumulation at stations where no DOC measurements were made during the ICESCAPE cruises, we took advantage of the small fraction of NCP that DOC accumulation represents and assumed a value of 7% of DIC drawdown in 2010 and 12% of DIC drawdown in 2011. Post-hoc comparisons of the calculated export fraction at stations where data were available revealed close agreement between the approach using an assumed percentage value and using the mixing-model derived value ($r^2=0.98$, slope=1 in 2010 and $r^2=0.87$, slope=0.8 in 2011). It should be noted that, regardless of the method used to estimate DOC accumulation, the overall conclusions derived from the results are not changed because of the uniformly low levels of autochthonous DOC production on the shelf.

Separating Stations by Flow Path / Region

As noted above, the summer waters of the Chukchi Sea shelf can be divided into warmer, nutrient-poor ACW, and colder, nutrient-rich BSW (e.g. Weingartner et al. 2005). The difference between these water masses is pronounced in sections from the ICESCAPE cruises (Figure 1-4, see also Lowry et al. 2015). Additionally, other waters are found on the shelf in the summer. This includes the recently ventilated winter water (WW) discussed above, which is taken here to be colder than -1.6°C , as well as remnant winter water (RWW), melt-water (MW), and Atlantic Water (AW). The RWW is winter water that has been warmed by solar heating and/or by mixing with summer waters.

We separate our analysis of C export from summer water on the Chukchi Sea shelf between stations that contained ACW in surface water and stations that had BSW or

any other water mass in surface waters, when occupied. Separations were made based on the T-S diagram shown in Figure 1-4. Thus, all stations were considered either as “ACW stations” or “non-ACW stations”. The vast majority of “non-ACW” stations had either BSW or MW when occupied. Water from the shelf flows through Barrow Canyon (BC) as it advects off of the shelf (Weingartner et al. 2005, Pickart et al. 2005, Figure 1-1). For each year we analyzed a set of stations, with both non-ACW and ACW, from transects located in this hydrographically complex BC region. Station designations (ACW, non-ACW) and the subset of BC stations are shown in Figures 1-5A and 1-5B for 2010 and 2011, respectively.

In 2010, POC data used to calculate C export were obtained from 79 stations located on the continental shelf (<150 m depth). Two stations were located near the

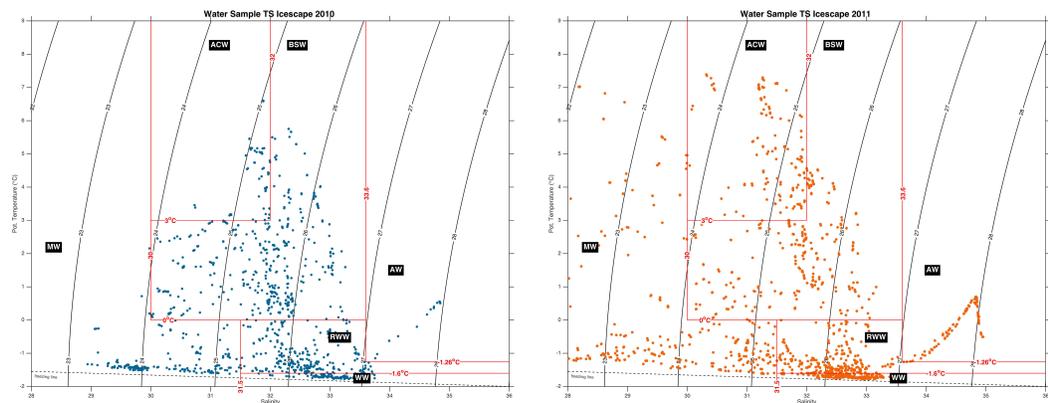


Figure 1-4. Temperature vs. salinity diagram for all depths and all stations sampled in 2010 and 2011. The boxes denote the approximate boundaries of the different water masses. ACW is Alaska Coastal Water, BSW is Bering Sea Water, RWW is Remnant Winter Water, WW is recently ventilated Winter Water, AW is Atlantic Water, and MW is Melt Water.

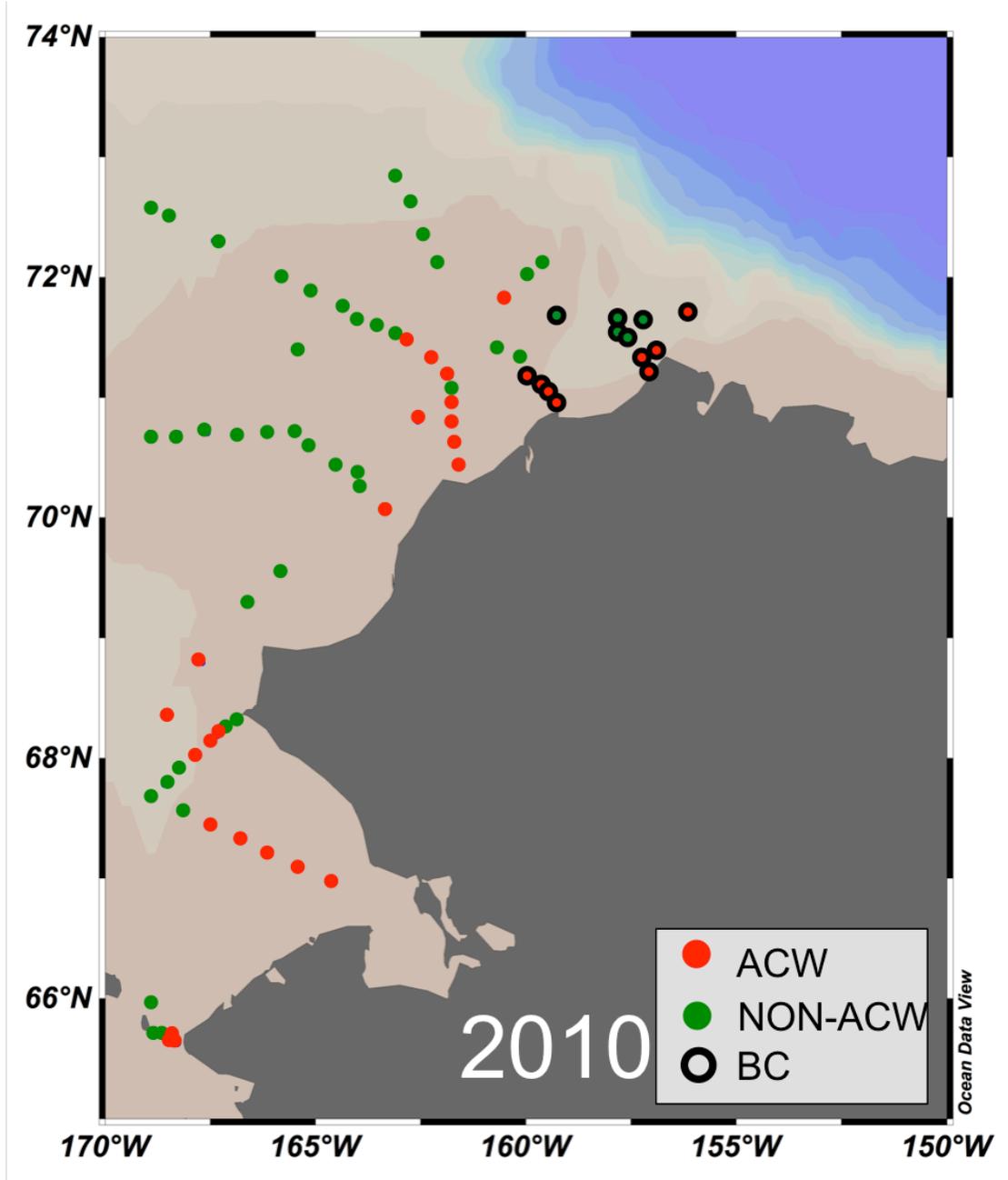


Figure 1-5A. 2010 stations for which mass-balance estimates were made using available DIC, POC and DOC data. ACW stations are shown in red. Non-ACW stations are shown in green. Barrow Canyon (BC) stations have dark circles.

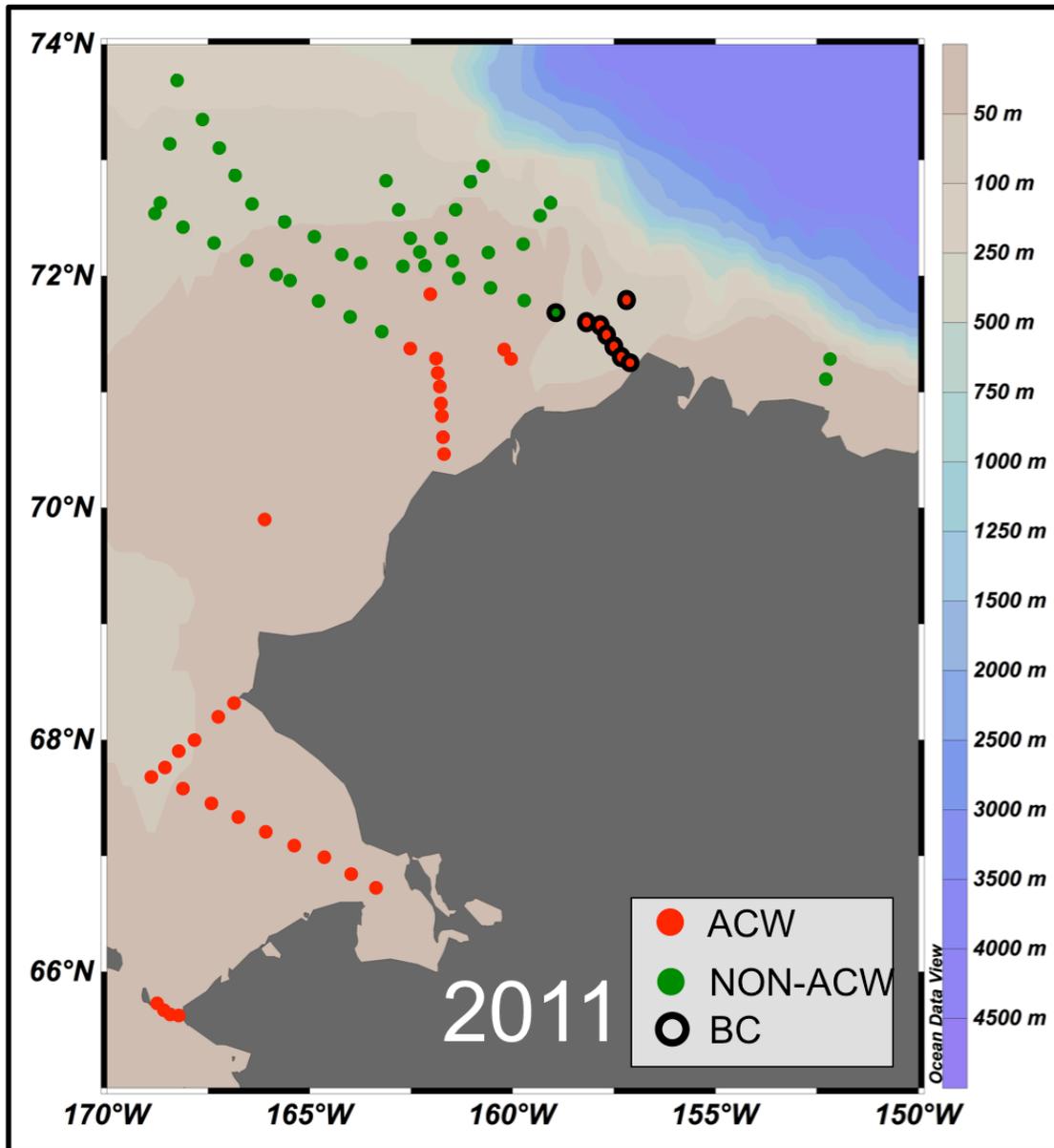


Figure 1-5B. 2011 stations for which mass-balance estimates were made using available DIC, POC and DOC data. ACW stations are shown in red. Non-ACW stations are shown in green. Barrow Canyon (BC) stations have dark circles.

head of Kotzebue Sound, where no ACW was observed in 2010. These two stations, likely subject to localized influences, were hydrographically distinct from stations with ACW or any other water-mass and were removed from the analysis. Of the 77 remaining stations, 32 stations were ACW stations, while 45 had non-ACW (either BSW or MW) when occupied. In 2010, a total of 13 stations were located on the Barrow Canyon Head, Barrow Canyon Center, or Barrow Canyon Mouth transects, which we consider to be in the BC hydrographic region. Of these, 8 stations exhibited ACW and 5 exhibited non-ACW.

In 2011, POC data used to calculate C export were obtained from 79 stations located on the continental shelf (<150 m depth). Of these 79 stations, 36 stations had ACW, while 43 had non-ACW (either BSW or MW). In 2011, a total of eight stations were located in the Barrow Canyon transects. Of these, seven stations exhibited ACW and one exhibited non-ACW.

All statistical analyses were performed using the R statistical software (<https://www.r-project.org>). P-values for comparisons between years or regions represent results of two-tailed t-tests, unless otherwise specified.

RESULTS

Runoff Fractions and DOC

We first present results from our mixing model approach used to estimating DOC_{ww} , needed to calculate export fractions. In 2010, the average runoff fraction was 8.6%, with a range from 0% (in AW) to 15.7% runoff (at several ACW stations). In 2011, the average runoff fraction for all stations was 8.5%, with a range from 0% (in

AW) to 20% runoff (at several ACW stations). As expected, DOC concentrations were negatively correlated with $\delta^{18}\text{O}$ (Figure 1-3A) and were strongly and positively correlated with runoff fraction (Figure 1-3B) in both years across the shelf. Using these relationships, the DOC concentration of shelf water, if it had no runoff water, would be $55.5 \mu\text{mol L}^{-1}$ for 2010 and $52.5 \mu\text{mol L}^{-1}$ for 2011. For near-average Arctic river water with a $\delta^{18}\text{O}$ value of -20‰, the apparent meteoric DOC concentration using the 2010 DOC: $\delta^{18}\text{O}$ relationship would be $219 \mu\text{mol L}^{-1}$, and using the 2011 relationship would be $190 \mu\text{mol L}^{-1}$. In 2010 and 2011, the average winter water, mixing-model-derived DOC concentrations were $68.8 \mu\text{mol L}^{-1}$ and $63.7 \mu\text{mol L}^{-1}$ DOC, respectively.

Mass Balance Estimates

DIC Deficits

The mean DIC deficit in the Chukchi Sea shelf water column across all stations at the time of sampling was $3516 \pm 2391 \text{ mmol C m}^{-2}$ in 2010 and $4197 \pm 2164 \text{ mmol C m}^{-2}$ in 2011. In both 2010 and 2011, ACW exhibited significantly greater DIC deficits than non-ACW ($p < 0.05$, see Table 1-1 and Figure 1-6). ACW DIC deficits were greater in 2011 than in 2010 by approximately $1000 \text{ mmol C m}^{-2}$, but these differences were not statistically significant ($p > 0.05$). This same trend held for BC stations, while DIC deficits in the canyon were unchanged between years in non-ACW (not shown).

Table 1-1. Estimated carbon export %, DIC deficit and POC accumulation by flow path for 2010 and 2011. Ranges represent one standard deviation around the mean.

2010 (n=77)	ACW (n=32)	Non-ACW (n=45)	Barrow Canyon Subset (n=13)
C Export %	65.6±24.4%	37.6±27.7%	36.9±30.5%
DIC Deficit	4423±2694 mmol C m ⁻²	2962±1972 mmol C m ⁻²	2824±2245 mmol C m ⁻²
POC Accumulation	882±542 mmol C m ⁻²	1591±1115 mmol C m ⁻²	1298±707 mmol C m ⁻²

2011 (n=79)	ACW (n=36)	Non-ACW (n=43)	Barrow Canyon Subset (n=8)
C Export %	75.5±7.0%	47.9±26.0%	67.2±10.8%
DIC Deficit	5447±2025 mmol C m ⁻²	3149±1677 mmol C m ⁻²	4790±3514 mmol C m ⁻²
POC Accumulation	645±358 mmol C m ⁻²	1063±664 mmol C m ⁻²	788±352 mmol C m ⁻²

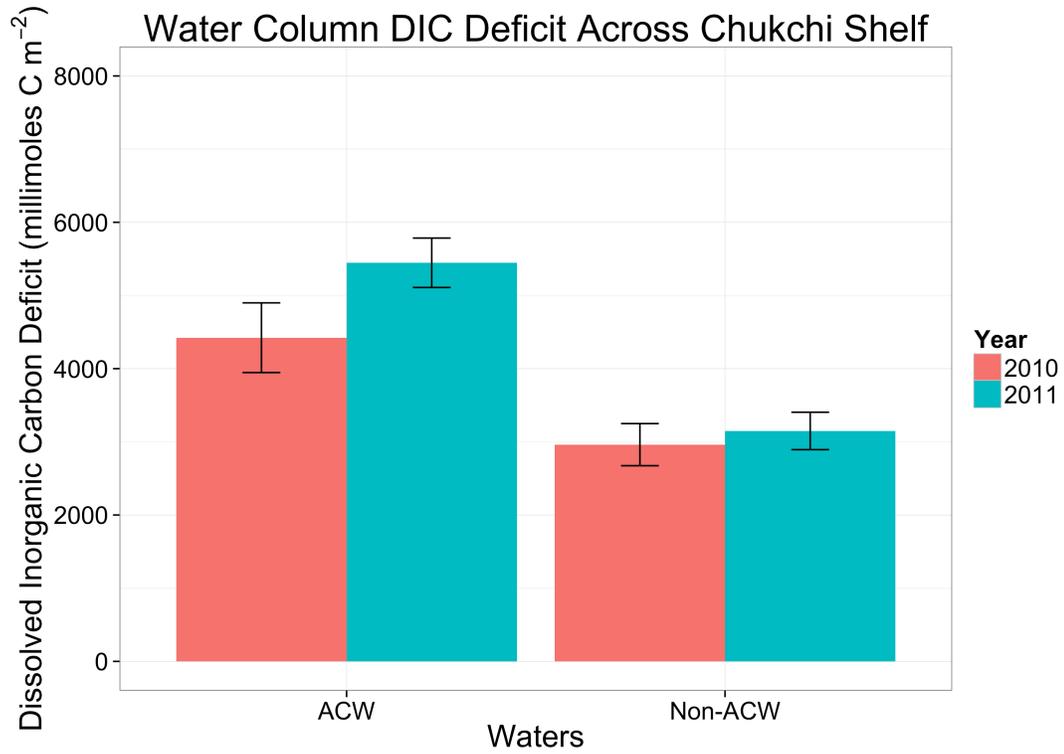


Figure 1-6. Dissolved inorganic carbon deficit (mmol C m⁻²) by cruise year and by water mass. Error bars are standard errors.

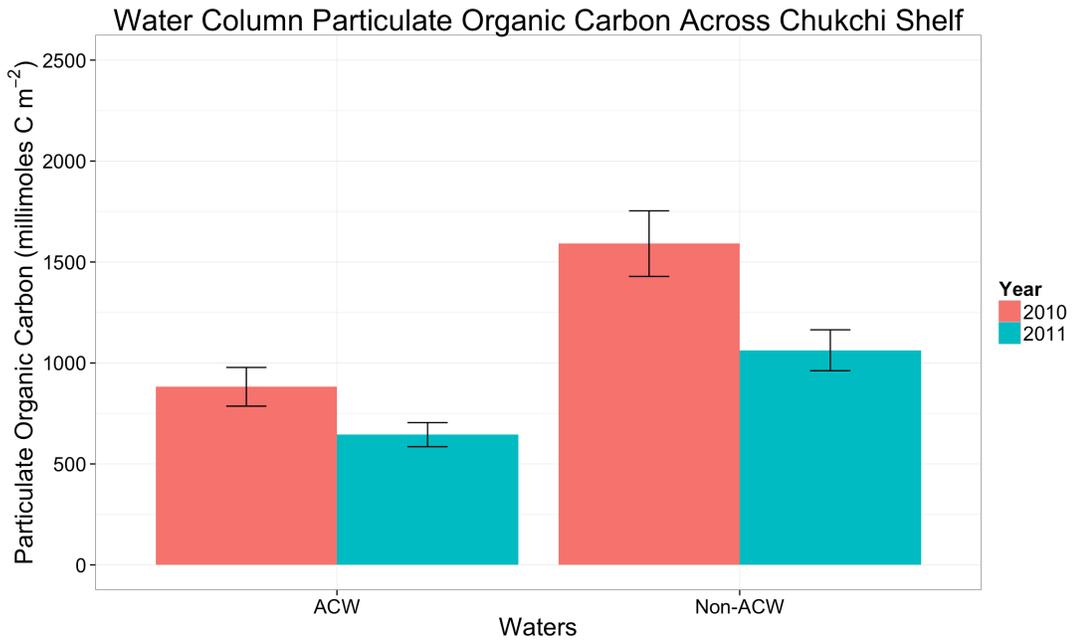


Figure 1-7. Estimates of water column particulate organic carbon (mmol C m⁻²) by cruise year and by water mass. Error bars are standard errors.

POC Accumulation

The mean POC accumulation in the Chukchi Sea shelf water column across all stations at the time of sampling was 1296 ± 977 mmol C m⁻² in 2010 and 872 ± 582 mmol C m⁻² in 2011. In both 2010 and 2011, POC accumulation in non-ACW was approximately double that in ACW ($p < 0.01$, see Table 1-1 and Figure 1-7). For both ACW and non-ACW stations, POC accumulation was significantly greater in 2010 than in 2011 ($p < 0.05$). Accumulation of POC at the BC stations in 2010 was 50% greater than in 2011 (Table 1-1).

Carbon Export

The mean mass-balance estimate of C_{org} exported from the water column across all stations at the time of sampling was $48.2 \pm 30.0\%$ ($n=77$, range 0-89%) and $60.4 \pm 24.0\%$ ($n=79$, range 0-83%) of total DIC drawdown in 2010 and 2011, respectively. Spatially averaged mass-balance estimates of the amount of C exported from the water column at the time of sampling were significantly greater in 2011 (2874 mmol C m⁻²) than in 2010 (2036 mmol C m⁻²) ($p < 0.05$). In both 2010 and 2011, there was a general trend of decreasing C_{org} export fractions from south-to-north across the shelf (Figures 1-8A and B).

In both 2010 and 2011, mean mass-balance estimates of C_{org} export from the water column were significantly lower in non-ACW than in ACW ($p < 0.01$; Table 1-1 and Figure 1-9). Among ACW stations, export percentages were greater in 2011 than in 2010 ($p < 0.05$). There was no difference in export fractions between 2010 and 2011

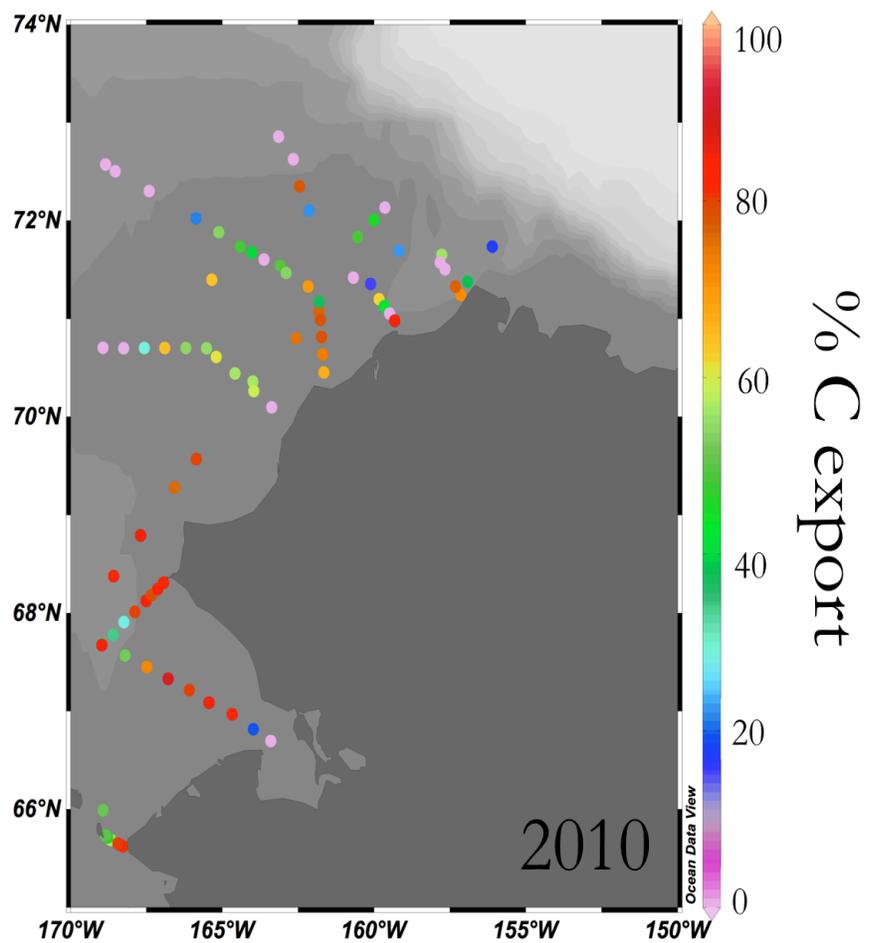


Figure 1-8A. Carbon export fraction estimates across the Chukchi Sea shelf in 2010.

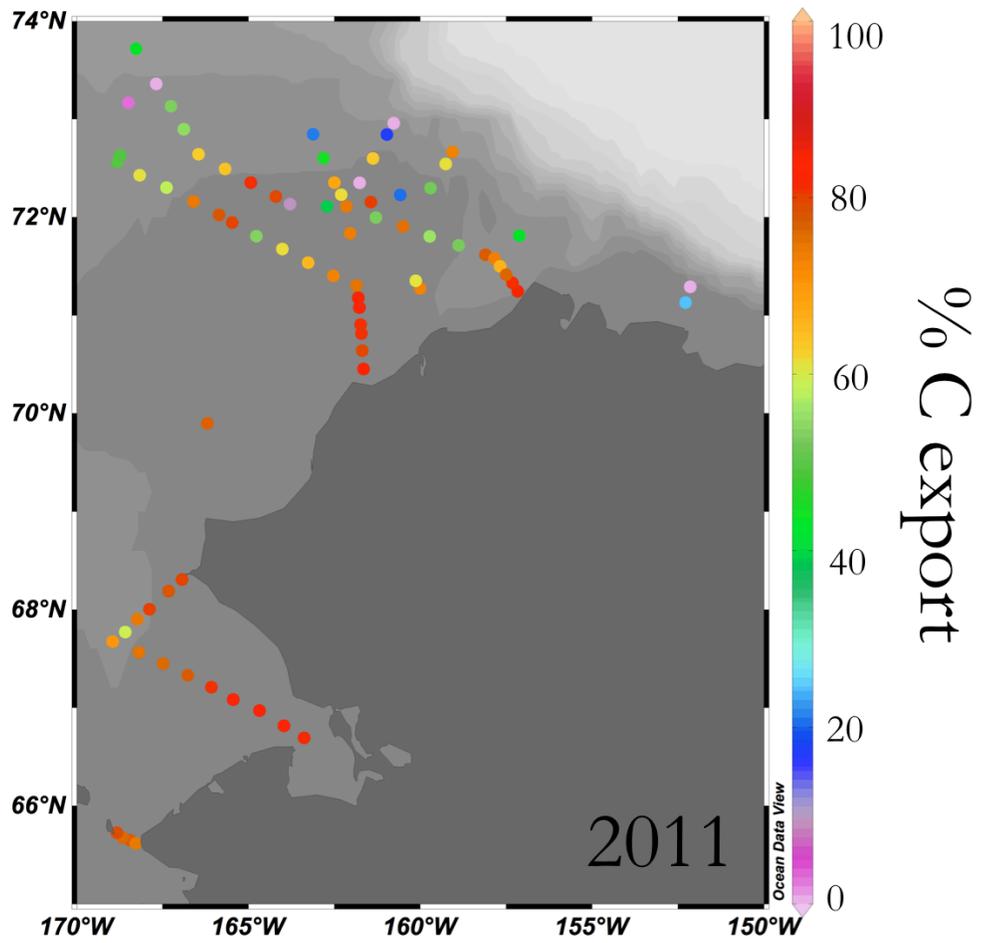


Figure 1-8B Carbon export fraction estimates across the Chukchi Sea shelf in 2011.

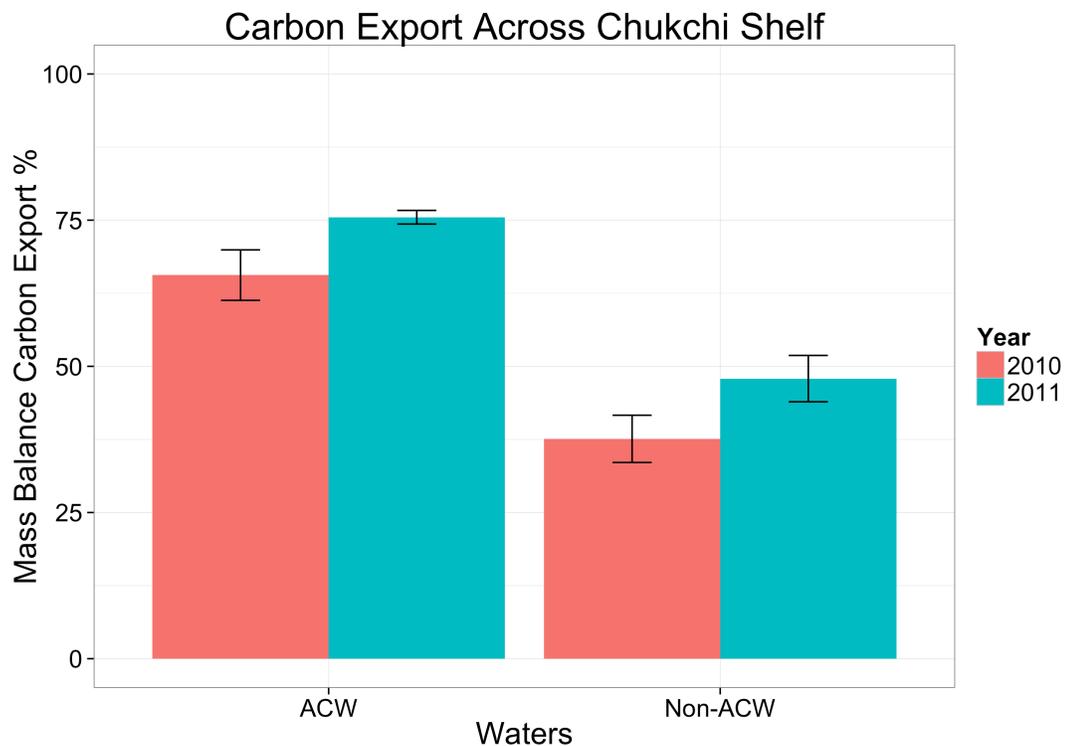


Figure 1-9. Estimates of carbon export % by year and by water mass. Error bars show standard error estimates.

among non-ACW. Stations located in BC showed inter-annual variability in the estimated export fractions. In 2010 (n=13 stations), BC had export fractions of $36.9 \pm 30.5\%$ of DIC drawdown, which was significantly lower than in 2011 (n=8 stations), when the export fractions were estimated to be $67.2 \pm 10.8\%$.

Export Relationship with Chlorophyll Concentration

We defined stations as having a phytoplankton “bloom” if a discrete water column measurement was $>1 \mu\text{g Chl a L}^{-1}$ (Lalande et al. 2011). Stations where the

near-surface discrete measurement (generally at 2 m depth) was $>1 \mu\text{g Chl a L}^{-1}$ were defined as having an active surface bloom, while stations with $<1 \mu\text{g Chl a L}^{-1}$ at the surface, but with at least one Chl a measurement $>1 \mu\text{g Chl a L}^{-1}$ at some greater depth were classified as having a sub-surface or sinking older bloom. Among all stations in 2010, there were 27 stations with active surface blooms, 39 stations with sub-surface or sinking older blooms, and 13 stations with no surface or sub-surface bloom. For 2010, the average C_{org} export fraction for stations with active surface blooms, sub-surface/sinking blooms, and no blooms was 29.5%, 54.1%, and 70.1%, respectively. In 2010, all stations that showed no sign of an active or recent bloom were in ACW, while all non-ACW stations exhibited evidence of a bloom, either at the surface or sub-surface. For 2011, the average C_{org} export fraction for stations with active surface blooms, sub-surface/sinking blooms, and no blooms was 51.1%, 59.1%, and 69.3%, respectively. In sum, for non-ACW stations with active blooms, or those that experienced recent blooms that were sinking at the time of sampling, C_{org} export fractions still ranged between 25-60% of drawdown by photosynthesis.

DISCUSSION

Primary production that sinks out of the water column in the shallow marginal seas of the Pacific Arctic is both ecologically and globally biogeochemically important. In addition to the mass balance approach used here, a variety of other approaches for estimating the fraction export of C from this shallow shelf system have been used. These include $^{234}\text{Th}/^{238}\text{U}$ disequilibria (Moran et al. 2005; Lepore et al. 2007; Lalande et al. 2007b) and drifting sediment traps (Lalande et al. 2007a) from BC and East

Hanna Shoal made as part of the SBI project, and NO_3^- utilization approaches, which relate the f-ratio (ratio of new production to total production) to C export (Hansell et al. 1993; Codispoti et al. 2013). Mathis et al. (2007b) employed a mass balance approach to estimate DOC and POC production as a fraction of total NCP in the northeastern Chukchi region, and Mathis et al. (2009) used a mass balance approach to estimate the fraction of NCP exported for this same region. Our approach estimates an instantaneous export fraction rather than an export rate, and our results are consistent with previously published ranges of C export fractions for the region (Table 1-2).

Most previous approaches have either focused on a particular region (BC and the east side of Hanna Shoal, e.g. Moran et al. 2005; Lepore et al. 2007; Mathis et al. 2007b; Mathis et al. 2009) or have taken a much broader, synthetic approach across the western Arctic region (Hansell et al. 1993; Codispoti et al. 2013). While Codispoti et al. (2013) does include winter measurements of nutrients in Bering Strait, all NO_3^- utilization and mass balance-based approaches on the Chukchi shelf suffer from the challenge of a paucity of winter water nutrient data for the region, meaning that annual budgets involve the significant assumption that winter water nutrient values are relatively uniform across years and across the shelf in order to perform temporal extrapolations.

Table 1-2. Comparison with published estimates of carbon export on the Chukchi Sea Shelf

Sources	Mean Export Estimates	Approach	Regional Coverage
Hansell et al. (1993) Data Synthesis	40-50%	f-ratio using NO ₃ ⁻ utilization	Chukchi Sea shelf, single value
Moran et al. (2005) Summer 2002	32% (20% exported off shelf)	²³⁴ Th/ ²³⁸ U disequilibrium and sediment traps	Northeastern Chukchi Sea shelf- and Barrow Canyon
Lepore et al. (2007) Summer 2002	15%	²³⁴ Th/ ²³⁸ U disequilibrium and sediment traps	Northeastern Chukchi Sea shelf- and Barrow Canyon
Lepore et al. (2007) Summer 2004	38%	²³⁴ Th/ ²³⁸ U disequilibrium and sediment traps	Northeastern Chukchi Sea shelf- and Barrow Canyon
Mathis et al. (2007b) Summer 2002	70%	Mass balance estimate as export production of NCP	Northeastern Chukchi Sea shelf- and Barrow Canyon
Mathis et al. (2009) Summer 2002	75%	Mass balance estimate as export production of NCP	Northeastern Chukchi Sea shelf
Mathis et al. (2009) Summer 2004	64%	Mass balance estimate as export production of NCP	Northeastern Chukchi Sea shelf
Codispoti et al. (2013) Data Synthesis	20%	f-ratio using NO ₃ ⁻ utilization	Chukchi Sea shelf, single value
This Study Summer 2010	48.2±30.0%	Mass balance using DIC deficit and organic carbon accumulation	Regionally specific values across Chukchi Sea shelf
This Study Summer 2011	60.4±24.0%	Mass balance using DIC deficit and POC/DOC accumulation	Regionally specific values across Chukchi Sea shelf

Our results are similar in magnitude to a previous study by Mathis et al. (2007b) that also used a mass-balance estimate approach to assess the fraction of NCP exported from the water column (Table 1-2). Our mixing-model derived winter water DOC concentrations were approximately 65 μM DOC, consistent with the pre-bloom concentration of $\sim 70 \mu\text{mol L}^{-1}$ DOC near Barrow Canyon (Mathis et al. 2007b), and our mixing model-derived estimates of the background DOC concentration in water with no runoff fraction are consistent with those reported by Cooper et al. (2005). The slope and intercepts of the relationship between DOC and $\delta^{18}\text{O}$ in summer 2010 and 2011 are similar to those from the May-June portion of the 2002 SBI cruise (Cooper et al. 2005), with some variation due to the fact that summer 2010 and 2011 data include changes to DOC concentrations due to autochthonous production during the growing season.

Taken as a whole, our results suggest that, given the assumption of a uniform mass of winter water, with constant pre-bloom DIC concentrations across the shelf, export percentages of over 50% of the DIC drawn down by phytoplankton production are common across the shelf. Our results also highlight that there is significant variability of C_{org} export fractions across the shelf. In both 2010 and 2011, export fraction ranged from 0% to roughly 80% across the shelf, meaning that, at some locations, nearly all the fixed C was exported out of the water-column, while at others, most of it remained in the water column in one form or another.

What drives spatial and temporal variability in C export?

North-South Gradient

Progressing northward across the shelf, fractions of C_{org} export generally decreased in both years. Some of this geographic variability could result from the timing of sampling relative to the timing of phytoplankton production. For example, at some more northern stations, phytoplankton were actively blooming at the time of sampling, and lower export fractions at these stations likely correspond with areas of greater water-column POC accumulation, which had neither been remineralized nor had time to sink.

In both years, all stations with active phytoplankton blooms at the surface had the lowest export fractions, followed by stations with sub-surface or sinking/ senescent blooms, while stations without any Chl a accumulation at any depth had the greatest C_{org} export fractions, averaging around 70% in both years. Any locations where phytoplankton had not yet bloomed should exhibit low DIC deficits and low water column POC (i.e., winter-like conditions). In both 2010 and 2011, there were almost no stations that had both low DIC deficits and low water-column POC accumulation, suggesting that those stations with higher export fractions, but no Chl a in the water column, likely experienced a bloom prior to sampling. Evidence for this is further supported by the high O_2 concentrations at many of these stations (Lowry et al. 2015).

Differences between 2010 and 2011

In ACW, regardless of whether or not phytoplankton were actively blooming, export fractions were significantly greater in 2011 than in 2010 ($p < 0.01$). This difference was driven by significantly greater water column POC in 2010 compared

with 2011 ($p < 0.01$), while DIC deficits were not significantly different between the two years. POC accumulation across all stations represented an average of 46% of the DIC deficit in 2010, while in 2011 POC only accounted for 28% of DIC deficit across all stations. The differences in average POC accumulation between 2010 and 2011 are likely due, in part, to the fact that in 2011 there were twice as many post-bloom stations sampled as in 2010, owing to the slight difference in seasonal timing of the cruises in the two years. However, among stations with active surface blooms, C_{org} export fractions were still significantly greater in 2011 (~50%) than in 2010 (~30%), suggesting that there is some inter-annual variability in the amount of C exported from the water column. These differences might be explained by differences in total NPP, changes in grazing pressure, or changes in phytoplankton species composition.

ACW vs. non-ACW Differences

In both 2010 and 2011, ACW had significantly greater C_{org} export fractions than non-ACW. This variability of export fractions between the ACW and non-ACW could potentially be an artifact of the winter water value assumptions used in our mass-balance calculation method. DIC deficits may be artificially inflated by the assumption of cross-shelf uniform winter water DIC values. If winter DIC values were significantly lower in ACW than was assumed here, then DIC deficits would be smaller than those calculated, and C_{org} export fractions lower. To examine this possibility, we calculated export fractions for ACW stations assuming the average DIC deficit of non-ACW. This recalculation reduced estimated export fractions calculated for ACW stations (from 65.6% to 59.8% in 2010 and from 75.5% to 58.8%

in 2011), but these recalculated values were still significantly higher than for non-ACW stations in both years. Thus, C export fractions remained greater in ACW stations than in non-ACW, even if we assume that the ACW exhibited the same DIC drawdown as non-ACW.

Pre-bloom DIC concentrations in ACW would need to be significantly lower, around 2050-2100 $\mu\text{mol L}^{-1}$ (rather than 2230-2270 $\mu\text{mol L}^{-1}$), in order for ACW to have the same calculated C export fraction as non-ACW (assuming POC accumulation remained unchanged). While such low pre-bloom DIC values may be possible (it is unknown given the lack of early season sampling in this region), our data nonetheless suggest that substantial export percentages (>50%) of any production that does occur in the ACW on the Chukchi Sea shelf are commonplace. The differences between the C_{org} export fractions in the ACW and non-ACW are fundamentally driven by the much lower accumulation of POC in the ACW. It should be noted that even though export percentages are much higher in the ACW than in the BSW flow paths, the amount of C exported from the water column in summer non-ACW, in terms of mol C m^{-2} , is two and half times greater on average than the amount exported from the ACW, due to the much higher rates of production in non-ACW.

Barrow Canyon

Following the trend for other regions, export fractions in the vicinity of BC were greater in 2011 than in 2010, due to very low POC accumulation in the water column in 2011. Barrow Canyon is thought to be a particularly productive region due to regular upwelling of deep basin waters (e.g. Aagaard and Roach, 1990) and the fact

that a number of winter water flow pathways across the Chukchi shelf converge there (e.g. Gong and Pickart, 2015; Lowry et al. 2015). Overall, observed export fractions in 2010 were more consistent with those observed in the region during the SBI project (Moran et al. 2005; Lepore et al. 2007), than the mass balance estimates made in 2011. All estimates exhibited large variability, which may be due to the inclusion of stations from a broad area around BC, but generally confirmed that BC is an important region of C export for the entire shelf system (Lalande et al. 2007a).

CONCLUSIONS

Our results demonstrate that strong pelagic-benthic coupling, with export percentages of >50% of fixed C_{org} , is common across the eastern Chukchi Sea shelf. In non-ACW with active blooms, or those that experienced recent blooms that were sinking at the time of sampling, export fractions still ranged between 25-60%, indicating an extremely rapid transfer of C_{org} from phytoplankton to the benthos, even during blooms. In both 2010 and 2011, ACW stations tended to have significantly higher fractions of C_{org} export than non-ACW stations, suggesting that the majority of fixed C quickly reaches the benthos in the ACW as well. Averaged across both years, stations with evidence of an earlier bloom, but where little Chl a and POC remained in the water column, had an average export percentage of 70% of NCP, with a POC accumulation around 20% and DOC accumulation around 10%. These percentages (70, 20, and 10%) for export, POC, and DOC, are identical to those reported by Mathis et al. (2007b) for the northeastern Chukchi Sea shelf. Nearly a decade later, as total NPP of the Chukchi Sea shelf appears to be increasing (e.g. Arrigo et al. 2011),

the highly productive regions of Chukchi Sea shelf ecosystem appear to be behaving similarly in terms of the rapid removal of fixed C from the water column. Notably, the less productive regions of the shelf exhibited very similar patterns of export to the more productive regions.

Continued changes in seasonal sea ice dynamics, including earlier retreat, are likely to have profound influences on both the timing and magnitude of phytoplankton production on the shelf (Grebmeier, 2012). The duration of seasonal sea ice cover on the Chukchi shelf has been decreasing in recent years and annual primary production has been increasing (Arrigo and van Dijken, 2011). Over two years of sampling at roughly the same time of year in this region, the variability in the fraction of C_{org} exported was most strongly controlled by the variability in the amount of POC in the water column, with less depth-integrated POC, and thus greater export fractions, in 2011 than in 2010. In particular, at stations where phytoplankton were blooming or had recently bloomed, export fractions were significantly lower in 2010 than in 2011, suggesting more rapid pelagic-benthic coupling in 2011. On the one hand, seasonally ice-free waters on the Chukchi Sea shelf have been associated with greater particulate organic matter flux relative to ice-covered waters (Lalande et al. 2007a). On the other hand, if sea ice retreats early enough, at a time of year when waters are colder, grazing rates may be very low and export (of ungrazed phytoplankton biomass) may increase (Grebmeier 2012). More work, including modeling efforts, and more years of data are needed to assess the long-term effects of changing sea ice retreat timing on the export of C from Chukchi Sea shelf waters.

Our results also demonstrate strong spatial and inter-annual variability in C export. Thus, while across the shelf >50% C_{org} export is common, eventually reaching values as high as 70% of DIC drawdown at the end of the phytoplankton growing season, there may be regions with much lower or much higher rates and these regions may change from year to year, perhaps depending on production, grazing rates, and sea ice dynamics and water temperature. While our results are at the high end of previous estimates of C_{org} export on the shelf, they are consistent with our understanding of the strong pelagic-benthic coupling of this system, and using a mass-balance approach, they take regenerated production from the water column (but perhaps not from the benthos) into account. Better estimation of pre-bloom winter water DIC concentration variability across the shelf will help constrain future estimates of export production, and will also shed light on the ultimate fate of the C_{org} after it has been exported to the benthos.

Chapter 2

Experimental fire increases soil CO₂ efflux in a grassland long-term, multi-factor, global change experiment²

ABSTRACT

Numerous studies have demonstrated that soil respiration rates increase under experimental warming, although the long-term, multi-year dynamics of this feedback are not well constrained. Less is known about the effects of single, punctuated events in combination with other longer-duration anthropogenic influences on the dynamics of soil carbon (C) loss. In 2012 and 2013, we assessed the effects of decadal-scale anthropogenic global change—warming, increased nitrogen (N) deposition, elevated carbon dioxide (CO₂) and increased precipitation—on soil respiration rates in an annual-dominated Mediterranean grassland. We also investigated how controlled fire and an artificial wet-up event, in combination with exposure to the longer-duration anthropogenic global change factors, influenced the dynamics of C cycling in this system.

Decade-duration surface soil warming (1-2°C) had no effect on soil respiration rates, while +N addition and elevated CO₂ concentrations increased growing-season soil CO₂ efflux rates by increasing annual aboveground net primary production (NPP) and belowground fine root production, respectively. Low-intensity experimental fire significantly elevated soil CO₂ efflux rates in the next growing season. Based on

² A version of this chapter, written with Tera P. Johnson, Nona R. Chiariello and Christopher B. Field, is currently under revision at *Global Change Biology*.

mixed-effects modeling and structural equation modeling, low-intensity fire increased growing-season soil respiration rates through a combination of three mechanisms: large increases in soil temperature (3-5°C), significant increases in fine root production, and elevated aboveground NPP. Our study shows that, in ecosystems where soil respiration has acclimated to moderate warming, further increases in soil temperature can stimulate greater soil CO₂ efflux. We also demonstrate that punctuated short-duration events such as fire can influence soil C dynamics with implications for both the parameterization of earth system models (ESMs) and the implementation of climate change mitigation policies that involve land-sector C accounting.

INTRODUCTION

At a planetary scale, soils represent a large stock of C, on the order of 2500 Pg C (Jobbagy and Jackson, 2000). The net loss or accumulation of soil C is a function of the balance between inputs of C from CO₂ fixation and losses from the ecosystem through respiration or physical soil removal. The net effect of anthropogenic global change on soil C cycling dynamics in an ecosystem is a function of the responses of each of these C cycle components (rates of NPP and rates of decomposition of litter and soil C over time). How much C will be removed from the atmosphere through CO₂ fertilization and incorporated into soils, and how much released to the atmosphere in response to warmer temperatures, elevated CO₂ concentrations, increases in extreme events, and other local-scale factors that characterize anthropogenic global change, is a key determinant in the trajectory of the Anthropocene.

At first order, rates of soil CO₂ efflux tend to increase, at least initially, with warmer temperatures (Raich and Schlesinger, 1992), due to a stimulation of microbial activity. Owing to this effect, Kirschbaum (1995) estimated that a 1°C rise in global temperature could lead to a loss of 10% of soil organic C in some regions (Kirschbaum, 1995), and recent meta-analyses have revealed a near-universal relationship between temperature and rates of soil respiration (Bond-Lamberty and Thomson, 2010; Mahecha *et al.* 2010). Despite these first order effects, the experimental evidence for responses of soil C to anthropogenic global change has been much more variable. In many ecosystems, soil respiration rates attenuate after a few years of experimental warming (Oechel *et al.* 2000; Luo *et al.* 2001), potentially due to an exhaustion of the labile soil C pool (Melillo *et al.* 2002) or to changes in carbon-use efficiency (Allison *et al.* 2010). Other studies have found no evidence of respiration acclimatization (Contosta *et al.* 2013).

At least part of the reason the aggregate feedback of soil C stocks to anthropogenic global change has been so difficult to characterize is that, in addition to increases in temperature and atmospheric CO₂ concentrations, anthropogenic global change is characterized by punctuated events and disturbances, of which several kinds are changing in frequency or intensity (IPCC, 2012). These extremes include heat waves, drought, and heavy precipitation events. Drought, warmer temperatures, and altered land management practices are increasing the prevalence and frequency of wildfire in some places (IPCC, 2012). Previous studies of the effects of wildfire on soil C dynamics have shown heterogeneous responses, including a depression of soil CO₂ efflux rates after wildfire in both a ponderosa pine forest (Sullivan *et al.* 2011)

and an arid grassland (Vargas *et al.* 2012). In both cases, fire was thought to oxidize labile C in the soils, suppressing future respiration.

Global change is also characterized by increasing variability and shifts in the seasonality of rainfall, which can strongly influence ecosystem C balance (Chou *et al.* 2008). Season-initiating wet-up in a seasonally dry Mediterranean climate can have strong influences on ecosystem functioning. There has been increased attention to the role of seasonal changes in driving pulses of soil CO₂ emissions (Borken and Matzner, 2009; Bowling *et al.* 2011; Nielsen and Ball, 2015). Understanding how the magnitude of the CO₂ pulse from seasonal wet-up interacts with other components of anthropogenic global change is helpful to characterizing the future seasonal dynamics of C cycling in Mediterranean ecosystems. Globally, characterizing ecosystem C balance responses under global change requires measurements of how inter-annual and seasonal variability, coupled with punctuated short-duration events such as wildfire, interact with longer-duration global change in their influence on soil CO₂ efflux. Estimating the response of soil CO₂ efflux to climate change remains a key challenge in the study of global change ecology, and, in particular, for constraining Earth System Models (Reichstein and Beer, 2008; Bond-Lamberty and Thomson, 2010; Subke and Bahn, 2010).

Characterizing the drivers of short-term, seasonal, and inter-annual variability in soil C loss is also important for effective climate change mitigation policy. Enhanced terrestrial C sinks, including those in soils, are likely to play a significant role in mitigating and offsetting anthropogenic emissions from fossil fuels as countries implement the 2015 Paris Agreement. The growing attention to managing ecosystems

for the service of C storage, and the active development of climate mitigation policies involving land-sector C accounting (e.g., California Air Resources Board, 2015), highlight the need for improved understanding of soil C cycle responses to anthropogenic influences.

Jasper Ridge Global Change Experiment

Over the last decade and a half, numerous studies at the Jasper Ridge Global Change Experiment (JRGCE) have investigated the response of an annual grassland ecosystem in central California, USA to elevated temperature, carbon dioxide, precipitation and nitrogen deposition in an effort to characterize ecosystem responses to anticipated anthropogenic influences (e.g., Shaw *et al.* 2002, Zavaleta *et al.* 2003, Dukes *et al.* 2011 *inter alia*). In this system, nitrogen addition has been found to stimulate above- and below-ground plant production (Dukes *et al.* 2005) and potential microbial decomposition (Gutknecht *et al.* 2010) but has had no effect on total microbial biomass C (Barnard *et al.* 2006). Experimental warming has had no effect, and elevated atmospheric CO₂ concentration has had no consistent effect on NPP in the JRGCE (Dukes *et al.* 2005). In a nearby grassland with similar plant species composition, elevated CO₂ increased rates of soil CO₂ efflux (Luo *et al.* 1996, Hungate *et al.* 1997) and induced N limitation of microbial decomposition at JRGCE (Hu *et al.* 2001). Both elevated precipitation and N addition increased the efflux of N₂O from soils (Brown *et al.* 2012). Rates of soil CO₂ production from incubated cored soils did not vary between any of the global change treatments (Niboyet *et al.* 2011b). As a whole, the ecosystem has exhibited the strongest NPP responses to N

addition, with variable responses to elevated CO₂ and nonlinear effects of warming and precipitation (Zhu *et al.* 2016), while soil microbial activity varies greatly across years (Gutknecht *et al.* 2012).

A low-intensity, accidental fire in 2003 provided an opportunity to assess the response of the soil biogeochemical cycling to wildfire. Niboyet and colleagues (2011a) found that fire greatly increased rates of N₂O emission from soils 2–3 years later, and exhibited a strong positive interaction with elevated CO₂ and N addition, thus representing a positive greenhouse-gas feedback to climate change (Niboyet *et al.* 2011a). The stimulation of N₂O production was likely due to increased anaerobic denitrification in burned soils (Niboyet *et al.* 2011a). Soil CO₂ efflux also increased in the second and third growing seasons after the fire, possibly driven by an increase in labile C substrate from either the concomitant increase in primary production or enhanced decomposition of soil organic matter (Niboyet *et al.* 2011a). In response to the accidental burn, NPP increased in burned plots that were also subjected to the +N addition, largely as a result of greater growth by annual grasses and forbs (Henry *et al.* 2006). The burn removed litter, leading to earlier seasonal growth in burned plots in the next growing season and increased soil temperatures by 4–6°C at the time of peak biomass (Henry *et al.* 2006).

Here, we provide a snapshot of the response of grassland soil CO₂ efflux to decadal-duration warming, as well as to an experimental wildfire and a simulated wet-up event, in combination with other longer-term global change factors at the Jasper Ridge Global Change Experiment.

METHODS

The Jasper Ridge Global Change Experiment (JRGCE) is a seventeen-year continuous (for each growing season) full-factorial global change manipulation with four principal treatments—elevated carbon dioxide (+275ppm CO₂), elevated temperature (+1–2°C at 1cm soil depth), increased precipitation (+50% above ambient rainfall), and elevated nitrogen deposition (+70 kg N ha⁻¹ y⁻¹ as Ca(NO₃)₂). JRGCE is located in a mostly annual grassland on clay-loam sandstone soil in central coastal California in the Jasper Ridge Biological Preserve, near Woodside, CA, USA (37°24' N, 122°14' W, 120m elevation). Typical of central coastal California, the site experiences a Mediterranean climate characterized by hot, dry summers and cool, wet winters. There is generally no, or only trace, rainfall between May and October. The average July daily maximum temperature is 31°C, and the average January daily maximum temperature is 16°C. The grassland community is dominated by non-native, naturalized annual grasses, principally *Avena barbata* and *Avena fatua*. Three species of native perennial bunchgrasses—*Elymus glaucus*, *Stipa pulchra*, and *Danthonia californica*—are also present. The vegetative growing season lasts six to seven months (November to May). Annual plants germinate and perennials re-green in late fall after wet-up from first growing-season initiating rains, generally in November. Senescence occurs after the onset of the dry season, commencing in late May. Peak plant community biomass occurs annually in late April to early May (Zavaleta et al. 2003).

The experimental design comprises eight replicated blocks, each of which contains four 2m-diameter circular plots, with each plot divided into four equal

quadrants. Within a replicate block, one quadrant receives each possible combination of the four global change treatments (Fig. 1). Thus, each factorial set is replicated eight times, once in each block. Temperature and elevated carbon dioxide are administered at the plot level (i.e., all quadrants in a plot receive the same treatment), whereas N application and water addition are conducted at the quadrant-scale (i.e., all quadrants in any plot receive a different combination of treatments).



Fig. 2-1. Map of Jasper Ridge Global Change Experiment area at the Jasper Ridge Biological Preserve. Circles represent 2m-diameter global change treatment plots (n=36 total). Orange circles indicate plots that receive both +CO₂ and heat treatments. Red circles indicate plots that receive heat treatment, but ambient CO₂. Yellow circles indicate plots that receive +CO₂ treatment, but ambient temperature. White circles indicate plots at ambient CO₂ and temperature. In blocks with two white circles, one is an infrastructure-free control. For each circular plot, one quadrant receives +N treatment, one quadrant receives elevated precipitation treatment, one quadrant receives both +N and elevated precipitation treatment, and one quadrant is held at ambient

precipitation and N. Red shading indicates the areas which were subjected to a low-intensity experimental burn in July 2011.

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indicate plots at ambient CO₂ and temperature. In blocks with two white circles, one is an infrastructure-free control. For each circular plot, one quadrant receives +N treatment, one quadrant receives elevated precipitation treatment, one quadrant receives both +N and elevated precipitation treatment, and one quadrant is held at ambient precipitation and N. Red shading indicates the areas which were subjected to a low-intensity experimental burn in July 2011.

In summer 2011, an additional, and this time intentional, treatment condition—a controlled burn—was applied to half the experimental blocks to provide a wildfire disturbance treatment (Fig. 1). Following Niboyet and colleagues (2011a) and Brown and colleagues (2012), measurements were made during the time of peak biomass in order to test the interactive effects of the global change treatments at a time of year when the effects of global environmental change on soil activity are likely to be most pronounced (Kammann *et al.* 2008). Intensive measurements at the time of peak biomass allow exploration of mechanisms underlying treatment effects but do not, in themselves, cover the implications of the treatments for annual carbon budgets, which can have complicated seasonal dynamics (Vicca *et al.* 2014).

Soil CO₂ Efflux Measurements

In the first and second years following the controlled burns, rates of soil CO₂ efflux were measured from nearly all quadrants at the approximate time of maximum aboveground plant biomass (April 27-29, 2012 and April 20-21, 2013), and after the onset of summer senescence (June 20-25, 2012 and June 25-July 15, 2013). One week

prior to the first set of measurements in April 2012, 10cm-diameter PVC soil collars were installed to a depth of approximately 5cm in all 128 quadrants. Soil collars were left in place and used for all subsequent measurements. One week prior to each set of measurements, all aboveground biomass within the collar was clipped to bare soil and removed. To ensure that the measured CO₂ fluxes were a result of belowground heterotrophic and autotrophic respiration, and not aboveground heterotrophic respiration, surface litter within the collar was removed.

Soil CO₂ efflux was measured using a closed-chamber method with a LiCOR LI-6400 portable photosynthesis system and LI6400-09 Soil Flux Chamber (LiCOR, Lincoln, NE). Before starting measurements, the LI-6400 was calibrated with a 422.7ppm CO₂ gas standard and zeroed using air passed through a soda lime scrubber. For each measurement, the soil flux chamber was placed immediately adjacent to the soil collar and allowed to equilibrate to ambient CO₂ concentrations for that plot (~385-400ppm CO₂ in plots without elevated CO₂, ~550-850ppm CO₂ in plots with elevated CO₂, depending on atmospheric mixing and wind speed). After the ambient atmospheric concentration was recorded, the chamber was placed onto the soil collar and sealed with a foam gasket. The previously measured adjacent ambient CO₂ concentration was set as a target concentration. Next, gas from within the chamber was pumped through a soda lime scrubber to reduce the within-chamber concentration by 15ppm CO₂ below the target. CO₂ within the chamber was then allowed to accumulate in the chamber until it reached 15ppm greater than the target. The amount of time for this increment was converted to an efflux rate in micromoles of carbon dioxide per meter squared per second ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), using the area of the soil

collar and volume of the soil chamber, including the measured depth of the installed soil collar. Efflux rates for each quadrant were recorded as the mean of three separate, successive measurements. All measurements were made between 8:30 and 17:30. Triplicate measurements of soil CO₂ efflux were made from 123 quadrants were made in April 2012 and from 89 quadrants in April 2013, for a total of 636 measurements. This procedure was repeated again in June 2012 and June 2013.

In order to avoid disturbing the soil adjacent to flux measurements, temperature data were collected hourly from previously installed in-situ thermocouples at 2cm depth immediately adjacent to the soil collar. Soil temperature at the time of measurement was taken as the value at the nearest hourly time-point to the measurement. In April 2012, 0-15cm and 15-30cm deep soil cores were taken from outside the soil collar, but within each quadrant, and soil moisture was assessed gravimetrically for each depth. Results are reported in percentages calculated as (wet soil mass (g)-dry soil mass (g)) / (dry soil mass (g)).

Total aboveground biomass and total belowground root biomass were part of the regular annual measurements at JRGCE. Total aboveground biomass was measured by clipping all aboveground biomass from a 141 cm² area within each quadrant, separating by functional group type (annual grass, annual forb, perennial grass, perennial forb), and drying at 70°C prior to weighing. Belowground plant biomass was measured for shallow (0-15cm depth) and deep (15-30cm) soil cores from which roots were removed, sorted into fine roots and taproots, dried and weighed.

Wet-Up Experiment

In order to examine the combined effects of a simulated wet-season initiating rainfall event, as would occur with the first rainfall at the end of the dry season, and nitrogen deposition on soil CO₂ efflux rates, 20mm of water was applied uniformly to the area within soil collars in early August 2013. The simulated rainfall event was intended to investigate the pulse of soil CO₂ efflux that occurs upon activation of the microbial community after a period of accumulation of rapidly decomposable materials during summer senescence. Post-senescence, dry-season soil CO₂ efflux rates were uniformly low, likely due to low soil moisture content.

The simulated rainfall event was applied to six quadrants with N addition (but all other global change factors at ambient levels) and to six control quadrants. In each of these quadrants, soil CO₂ efflux rate was measured immediately prior to wet-up, immediately after wet-up, and at 1, 2, 4, 6, 24 and 48 hours post wet-up. In order to assess changes in soil moisture due to the simulated rainfall event, we replicated the 20mm treatment in plots of bare soil near the plots and measured soil moisture gravimetrically from the top 5cm at 0, 1, 2, 4, 6, 24, and 48 hours after wet-up.

Analyses

Analyses were organized hierarchically. First, we developed a linear mixed-effects model to estimate the effects of the five treatments on rates of soil CO₂ efflux for both April 2012 and April 2013, and then with a model combining data from both years. Second, we adopted a path analysis approach using structural equation modeling, to help elucidate the role of soil temperature, nutrient availability, and plant

community attributes as both direct and indirect drivers of soil CO₂ efflux.

The linear mixed effects model includes all five treatments and all interaction terms, with soil CO₂ efflux rate as the dependent variable. All treatment effects on soil CO₂ efflux were estimated using restricted maximum likelihood estimation. Both treatment and interaction terms were iteratively removed from the model based on Akaike Information Criterion (AIC)-based selection to arrive at the maximum likelihood linear model for estimating soil CO₂ efflux rates.

To address non-independence of the treatments nested within plots, we included a random effect for plot. This allows us to assess and remove the effects of plot-to-plot differences by allowing a different baseline efflux rate (intercept) for each plot. Measurements from two quadrants in a single plot had a correlation coefficient of 0.38 in April 2012 and 0.22 in April 2013.

Model selection was performed first using the Linear Mixed Effects (`lme`) function in the **nlme** package in R, version 3.1-117 (Pinheiro *et al.* 2012), and repeated using the Fit Linear Mixed Effects Models (`lmer`) function in the **lme4** package in R, version 1.1-6, (Bates *et al.* 2014), as a check for consistency of model performance across packages with slightly varying statistical assumptions. Model code is presented in the supplementary information.

In order to elucidate possible mechanisms by which the global change treatments might be influencing soil CO₂ efflux rates, we used the results of the linear mixed-effects model analysis in a structural equation modeling approach. Both environmental variables (soil moisture and soil temperature), and the attributes of grassland plant community (aboveground biomass, belowground biomass fractions,

and litter biomass, both from that growing season and from the previous growing season) may be responding to the global change treatments, but may also be directly influencing soil CO₂ efflux rates. Thus, these environmental variables and plant community attributes can act as both response (to the global change treatments) and predictor (of CO₂ efflux rates) variables. Because the goal of this study was to understand how soil respiration responds to global change, environmental conditions and plant community attributes were treated as mediator variables, capable of both responding to the experimental treatments, and also controlling the dynamics of our response variable of interest—soil CO₂ efflux.

We began with an *a priori* conceptual model in which (1) all of the plant community and environmental variables that responded to any of the five global change factors were included as mediator variables, and (2) soil CO₂ efflux responded to these environmental conditions and plant community attributes, as well as to all five global change treatment factors (Fig. 2a). This “all inclusive” initial conceptual model was designed to assess whether and how plant community and environmental variables were associated with soil CO₂ efflux rates. For example, if the effects of N addition on soil CO₂ efflux strongly co-varied with specific plant community attributes, this might suggest a mechanism by which N enrichment influences soil CO₂ efflux. However, if no covariance was present, this would suggest direct influence of the experimental treatment, unmediated by the plant community or undetectable based on available environmental measurements. The model was developed using the **lavaan** package in R, version 0.5-117. (Rosseel *et al.* 2012). We evaluated the fit of this initial model using maximum likelihood estimation in the **sem** function within the **semTools**

package, version 0.4-0 (Pornprasertmanit *et al.* 2013). All non-significant path coefficients were removed, resulting in the final path diagram model (Fig. 2b). R version 3.0.2 (September 25, 2013) was used for all analyses. All structural equation model code is available in the supplemental materials.

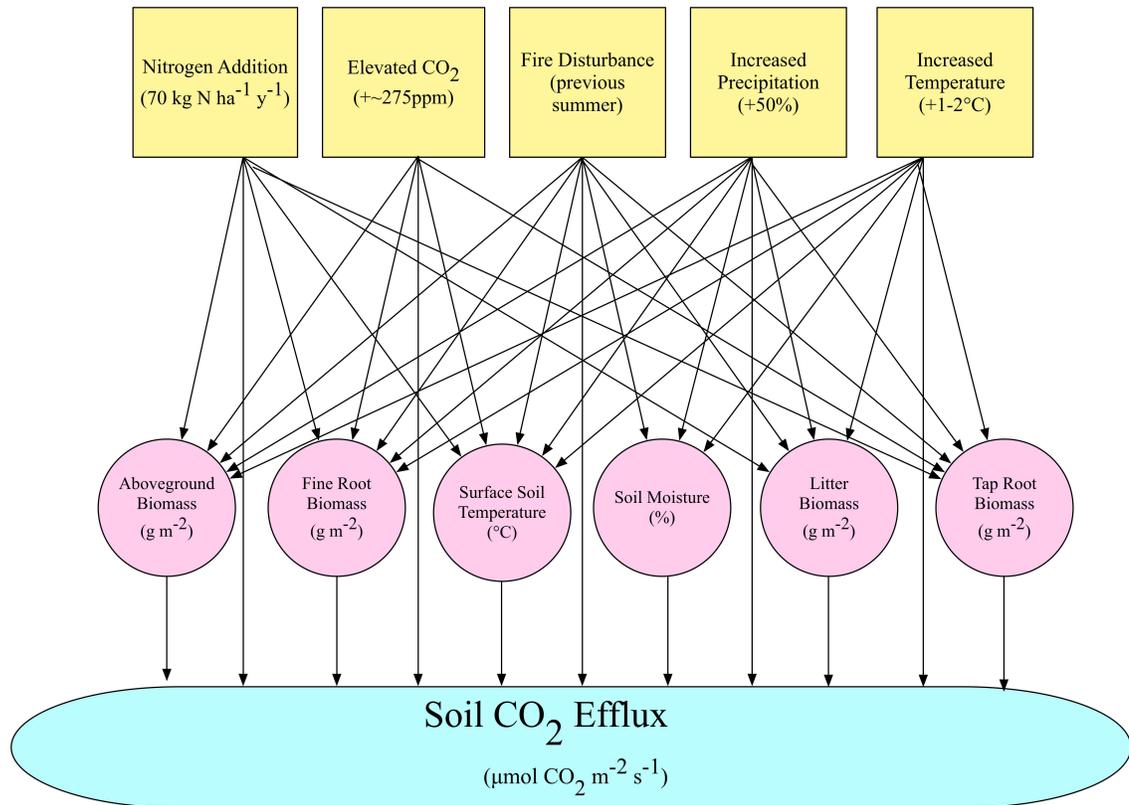
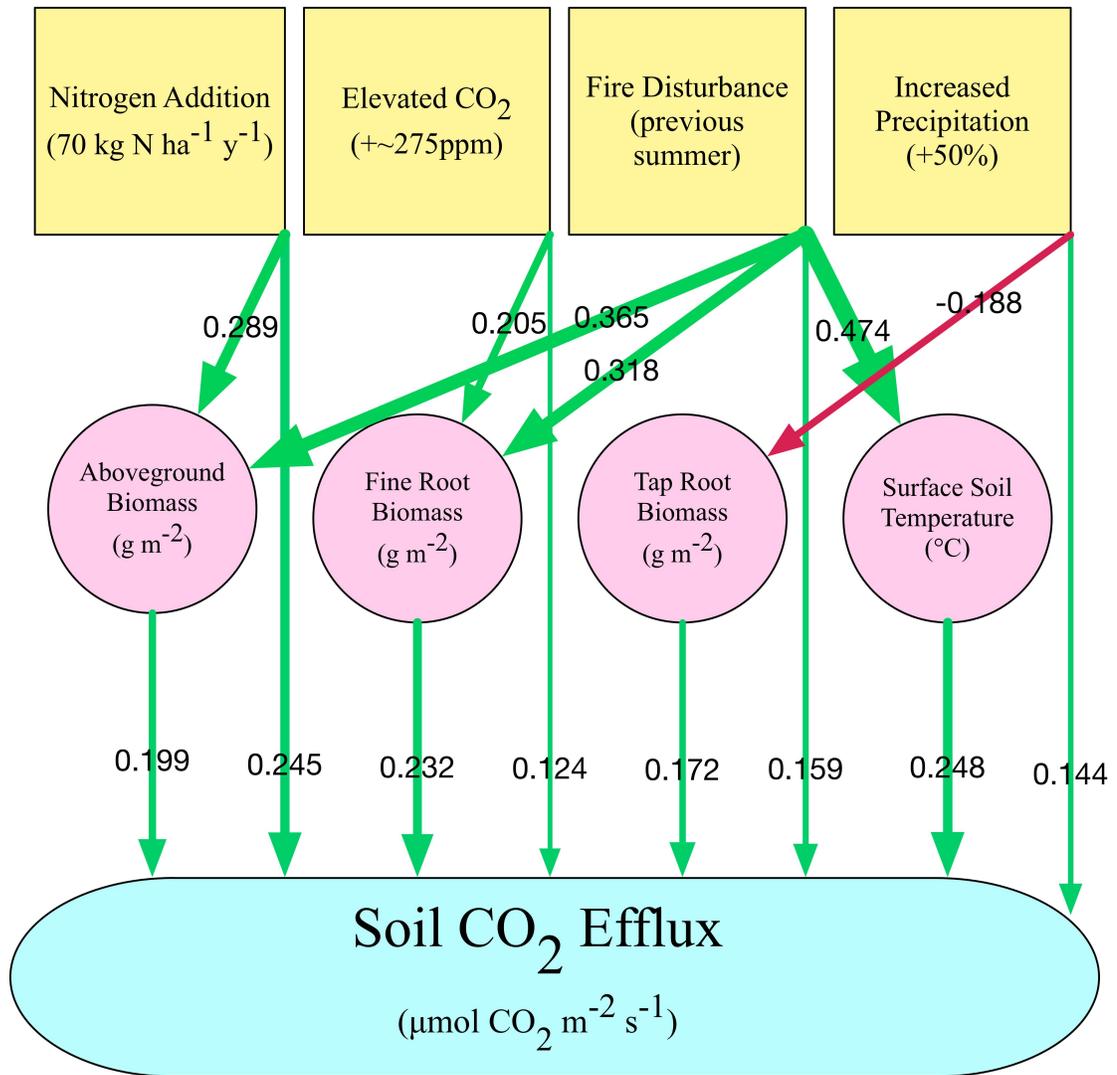


Fig 2-2A. Initial *a priori* structural equation model showing all possible direct and indirect effects of all five global change treatments and mediator variables on soil CO₂ efflux rates.



Model $R^2 = 0.47$

Fig. 2-2B. Final structural equation model showing all significant path coefficients for both direct and indirect effects of global change treatments and mediator variables on soil CO_2 efflux rates. Positive correlations are shown as green arrows and negative correlations are shown as red arrows, and the width of the arrow is proportional to the strength of the correlation. Global change treatments are shown in yellow, the primary response variable is shown in blue and the mediator variables are shown in pink. The standardized path coefficients and total model R^2 are also given. Note that for the elevated precipitation treatment, there is a direct positive effect on soil CO_2 efflux that is masked because of the negative influence of elevated precipitation on tap-root biomass, which is positively correlated with CO_2 efflux.

Previous studies have found that nutrient availability responds to global change factors (Hyvonen *et al.* 2007) and can influence rates of CO₂ efflux (Janssens *et al.* 2010). Measurements of soil nutrient data (NH₄⁺, NO₃⁻, PO₄³⁻) were made within a small subset of plots (in 16 of the 128 total quadrants) in April 2012. Because of the low sampling coverage of our nutrient dataset, we did not include nutrient availability as an intermediate variable in the structural equation modeling presented here. However, we did develop a separate structural equation model for the 16 quadrants for which we have fully available nutrient data. That model and its results are presented in full in the supplemental materials.

In order to avoid the potential for Type I errors, we report statistical comparisons of significance in the responses of environmental variables (soil CO₂ efflux, soil moisture, soil temperature) and plant community attributes (aboveground biomass, belowground biomass, fine root biomass, litter biomass, deep root biomass, shallow root biomass) to global change treatments based on Bonferroni correction of p-values for multiple comparisons. The results of linear mixed-effects models are reported based on a conservative restricted maximum likelihood model, including t-value, p-value and degrees of freedom.

RESULTS

Soil Temperature

In April 2012, soil CO₂ efflux rates were positively correlated with soil temperature at the time of measurement, across all treatment types (simple linear regression F=16.85, d.f.=121, p<0.001). Based on a quadratic fit of the data, the Q₁₀ of

the response of soil CO₂ efflux to temperature was 2.82. In April 2012, at the time of measurement, soil temperature at 2cm was approximately 5°C warmer in burned (24.6°C) than in unburned plots (19.6°C) ($t = 5.8577$, $df = 101$, Bonferroni adjusted p -value <0.001). Nutrient addition had no effect on soil temperature at the time of measurement, (+N quadrants: 21.4°C; ambient N quadrants: 22.7°C). Consistent with previous studies at JRGCE (Niboyet *et al.* 2011a), near-surface soil temperatures were 1.1°C higher in heated plots relative to ambient temperature plots. The difference in temperature between burned and unburned plots was not simply a function of the temperature at the time-of-day of measurement or a transitory response at the time of measurement. The two-week mean daily soil temperature in burned plots (23.9°C) was greater than in unburned plots (21.0°C) by nearly 3°C in April 2012 (Bonferroni adjusted $p < 0.01$).

Soil Moisture

At the time of CO₂ efflux measurements in 2012, gravimetric soil moisture in the top 15cm of the soil averaged 14.6±3.3% across all plots. Soil moisture was greater in the burned (15.6±2.4%) compared to the unburned plots (13.6±3.8%, $t=3.5226$, $d.f.=105$, Bonferroni adjusted $p < 0.01$) and was lower in warmed plots (13.7±4.3%) relative to unwarmed plots (15.5±1.3%, Bonferroni adjusted $p < 0.05$). The elevated precipitation treatment marginally increased soil moisture (15.4±3.5%) relative to ambient precipitation (13.8±2.9%, $t=2.63$, $d.f.=117.26$, Bonferroni adjusted $p < 0.08$). Soil moisture exhibited no differences between the +N and ambient N plots (Bonferroni adjusted $p=1.00$) or the CO₂-enriched and ambient CO₂ plots (Bonferroni

adjusted $p=1.00$). Across all treatments, average soil moisture was highest in plots that were burned the previous summer and exposed to increased precipitation ($16.5\pm 2.8\%$). This is 3.6% more than in plots that were not burned and had ambient precipitation ($12.9\pm 3.6\%$).

In April 2013, two years after the burn, soil moisture values were much lower overall ($7.3\pm 2.0\%$) than in April 2012 ($14.6\pm 3.3\%$), likely owing to the ongoing drought in California. In April 2013, the warming treatment again decreased soil moisture relative to ambient ($t=4.38$, d.f.= 120, Bonferroni adjusted $p<0.01$). No other global change factor, including the elevated precipitation treatment, nor the 2011 burn, had measured effects on soil moisture in 2013 (Bonferroni adjusted $p=1.00$).

Biomass and Litter

In April 2012, average total aboveground biomass was greater in burned (463 ± 246 g m⁻²) compared to unburned plots (288 ± 197 g m⁻², $t=4.32$, d.f. =112, Bonferroni adjusted $p<0.01$) and was greater in +N plots (444 ± 268 g m⁻²) compared to ambient N plots (304 ± 182 g m⁻², $t=3.35$, d.f.=105, Bonferroni adjusted $p<0.02$). There were no differences in aboveground biomass as a result of any other global change treatment (Bonferroni adjusted $p=1.00$ for all treatments).

Total root biomass in the top 15cm of soil in April 2012 was weakly, but positively correlated with soil CO₂ efflux rates (simple linear regression: $R^2=0.05$, $F=5.74$, d.f.=121, $p<0.02$). This relationship was absent in April 2013. In April 2012, total root biomass in the top 15cm of the soil profile was decreased by added precipitation relative to plots receiving ambient precipitation (Bonferroni adjusted

$p < 0.01$). It was not affected by fire, N-enrichment, or warming (Bonferroni adjusted $p = 1.00$).

Fine root biomass (but not taproot biomass) in the top 15cm of soil was positively correlated with soil CO₂ efflux ($R^2 = 0.14$, $F = 21.02$, d.f. = 121, $p < 0.001$). And in contrast with total root biomass, low-intensity fire greatly increased fine root biomass compared to unburned controls ($t = 3.68$, d.f. = 103, Bonferroni adjusted $p < 0.01$). Elevated CO₂ increased fine root biomass ($t = 2.35$, d.f. = 120, Bonferroni adjusted $p = 0.18$), but this effect was only marginal after performing the restricted test for multiple comparisons. Added precipitation, which increased total root biomass, by contrast, had no effect on fine root biomass (the entire effect was due to increases in tap root biomass). Thus, fire increased fine root biomass (with an additional positive effect of elevated CO₂), and elevated precipitation increased taproot biomass.

Litter mass was roughly 10x greater in unburned plots ($210 \pm 143 \text{ g m}^{-2}$) relative to burned plots ($20 \pm 23 \text{ g m}^{-2}$) in April 2012 ($t = 10.37$, d.f. = 65, Bonferroni adjusted $p < 0.001$). Despite greater aboveground biomass production in 2012 in burned plots, litter mass in April 2013 continued to be greater in unburned plots ($194 \pm 87 \text{ g m}^{-2}$) relative to burned plots ($148 \pm 84 \text{ g m}^{-2}$) ($t = 3.01$, d.f. = 118, Bonferroni adjusted $p < 0.02$), two growing seasons after the burn.

Soil CO₂ Efflux

Long-term warming had no direct effects on soil CO₂ efflux rates in April 2012 or April 2013 (Bonferroni adjusted $p = 1.00$) and exhibited no interactions with the other global change treatments. Soil CO₂ efflux rates in April 2012 were not different

between long-term elevated precipitation and ambient-precipitation plots (Bonferroni adjusted $p=1.00$).

Long-term N addition increased soil CO₂ efflux rates in April 2012 (t-test: $t=3.04$, d.f. =119, Bonferroni adjusted $p < 0.03$; 2012 linear mixed effects model: $t=4.18$, d.f.= 90, $p < 0.001$, Table 1). In April 2012, elevated atmospheric CO₂ had a marginally significant positive effect on soil CO₂ efflux (t-test: $t=2.15$, d.f.=120, Bonferroni adjusted $p < 0.29$; 2012 linear mixed-effects model: $t=1.78$, d.f. =29, $p < 0.08$) but improved the overall fit of the mixed effects model, based on AIC.

Fire disturbance increased soil CO₂ efflux at the time of peak aboveground biomass (t test: $t=5.30$, d.f. = 116, Bonferroni adjusted $p < 0.001$; 2012 linear mixed-effects model: $t=3.97$, d.f. 29, $p < 0.001$) in April 2012, but this effect lasted only one growing season. The fire had no effect on efflux rates in April 2013, two growing seasons after the fire disturbance (Bonferroni adjusted $p = 1.00$, and see mixed effects model results). At the peak of the first growing season (April 2012), there was no significant interaction effect between any of the treatments. The positive effects of the fire and N addition on soil CO₂ efflux were additive (Fig. 3).

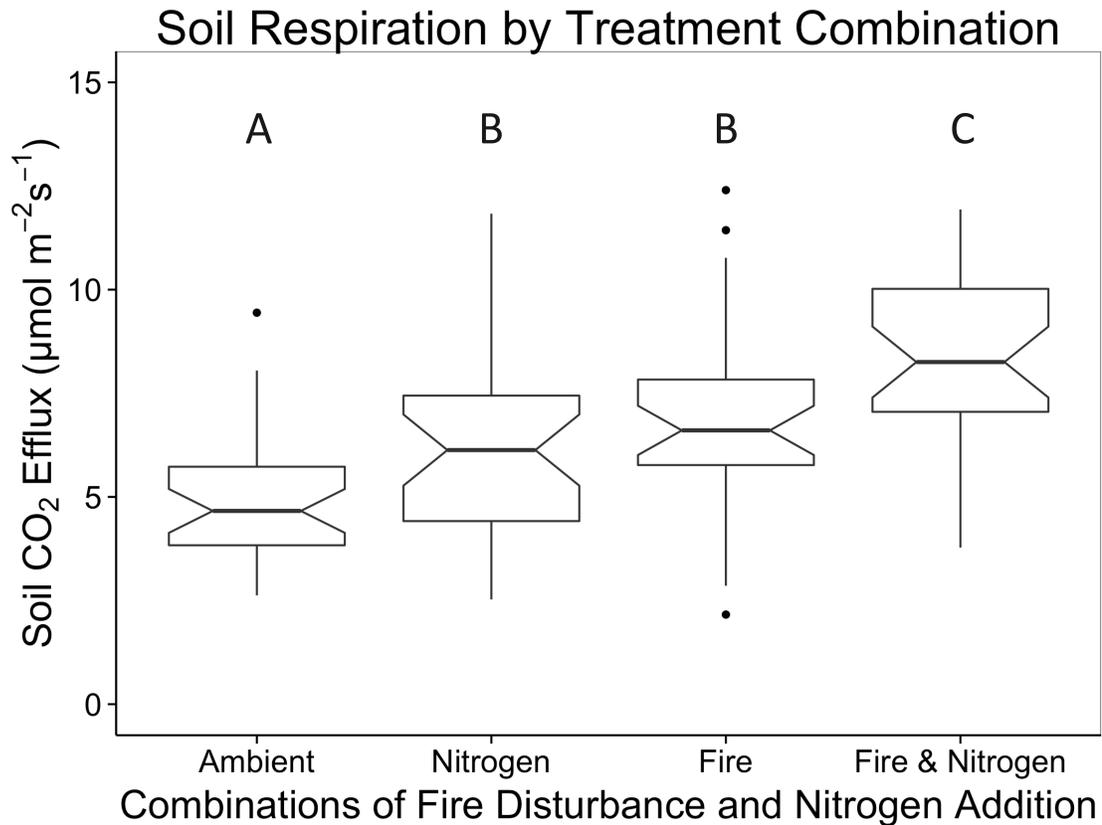


Fig. 2-3. The effects of nitrogen enrichment and fire disturbance on soil CO₂ efflux rates. Each boxplot shows the full-distribution of data. The median is the thick black line, edges of the boxes represent the first and third quartiles. Each box is generated from measurements of soil CO₂ efflux from 32 quadrants per treatment combination, averaging values across ambient and elevated temperature, CO₂, and precipitation. Letters indicate significant differences.

In April 2012, fire increased soil carbon dioxide efflux by nearly 2.1 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, or approximately 36% relative to unburned controls (mixed effects model: $p < 0.001$, Table 1). In April 2012, +N increased soil CO₂ efflux by 1.2 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, or 20% relative to ambient N controls (mixed effects model: $p < 0.001$, Table 1). In April 2013, N addition marginally increased soil CO₂ efflux by 1.1 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, or 19% relative to controls (mixed effects model: $p < 0.12$, Table 1).

For April 2012, information criterion-based model selection resulted in a linear mixed effects model with the form:

$$\text{EFFLUX}_{ij} = \beta_0 + \beta_1 \text{BURN}_{ij} + \beta_2 \text{NITROGEN}_{ij} + \beta_3 \text{CO}_{2ij} + a_i + \varepsilon_{ij}$$

where EFFLUX_{ij} is the soil CO_2 efflux rate for observation j in plot i , BURN_{ij} , NITROGEN_{ij} and CO_{2ij} are nominal variables with two levels (ambient and treatment). The term a_i is a random intercept and is assumed to be normally-distributed with mean 0 and variance d^2 . The residual term, ε_{ij} , is assumed to have mean 0 and variance σ^2 . β_0 is the fixed intercept term, while β_1 , β_2 , and β_3 represent the estimated effects of each of the global change treatments. Terms for elevated temperature (HEAT_{ij}) and precipitation (WATER_{ij}) were dropped from the model. Examination of residuals showed that both assumptions for the distributions of a_i and ε_{ij} were met. The 2012 model had a marginal R^2 value of 0.29, meaning 29% of the variance in efflux rate is explained by the fixed effects of the global change treatments, and a conditional R^2 value (*sensu* Nakagawa and Schielzeth, 2013) of 0.56. Model coefficient estimates are presented in Table 2-1.

For April 2013, information criterion-based model selection resulted in a linear mixed effects model with the form:

$$\text{EFFLUX}_{ij} = \beta_0 + \beta_1 \text{NITROGEN}_{ij} + \beta_2 \text{CO}_{2ij} + a_i + \varepsilon_{ij}$$

Examination of residuals showed that both assumptions for the distributions of a_i and ε_{ij} were met. The 2013 model had a marginal R^2 value of 0.06 and a conditional R^2 value of 0.28 (Nakagawa and Schielzeth, 2013). Coefficient estimates are presented in Table 2-1. Overall, the fit of the model was much better in 2012 than in 2013, when efflux rates were more variable.

After senescence and dry-out in summer 2012 and 2013, soil CO_2 efflux rates were lower and more variable than during peak biomass ($\sim 1.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ vs. $<$

~5-12 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). For many quadrants, no detectable soil CO_2 efflux could be measured after 30 minutes (i.e. flux was 0). There was no significant effect of any of the global change treatments during the summer period, including between burned and unburned plots, in either 2012 or 2013. This diminished rate of soil CO_2 efflux is attributable to extremely low soil moisture content (<5%) during summer measurements.

Given that these two mixed effects models reveal consistent effects of +N and + CO_2 treatments on soil CO_2 efflux and a strong, but temporary effect of the +Burn treatment lasting only one year, we developed a combined single linear mixed-effects model that included time as a factor and interaction terms between time and each of the significant global change treatment factors. For the combined model, with year of measurement as a factor, information criterion-based model selection resulted in a linear mixed-effects model with the form:

$$\text{EFFLUX}_{ij} = \beta_0 + \beta_1 \text{BURN}_{ij} + \beta_2 \text{NITROGEN}_{ij} + \beta_3 \text{CO}_{2ij} + \beta_4 \text{YEAR}_{ij} + \beta_5 \text{YEAR} * \text{BURN}_{ij} + a_i + \varepsilon_{ij}$$

This model allows us to assess the consistency of the effects of the +Nitrogen and + CO_2 treatments across years, while also tracking the interactive effects of the +Burn and time. Coefficient estimates for the combined model are presented in Table 2-2. The model had a marginal R^2 of 0.16 and a conditional R^2 of 0.27. Overall, the combined model shows that the +Burn treatment's positive effect on soil CO_2 efflux disappears in 2013, while the positive effects of +N and + CO_2 remain across years.

Table 2-1. Linear Mixed Effects Model parameter estimates for significant global change treatment effects on soil CO₂ efflux rates measured in April 2012 (one year after fire disturbance) and April 2013 (two years after fire disturbance).

April 2012 Model				April 2013 Model		
<i>Parameter</i>	<i>Estimate</i> ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	<i>DF</i>	<i>P</i> <i>value</i>	<i>Estimate</i> ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	<i>DF</i>	<i>p-value</i>
Fixed intercept (β_0)	4.48±0.48	90	<0.001***	4.77±0.58	56	<0.001
+Burn treatment	2.05±0.53	29	<0.001***			
+Nitrogen treatment	1.23±0.53	90	<0.001***	1.07±0.67	56	<0.122
+CO₂ treatment	0.92±0.29	29	<0.10*	1.23±0.67	56	<0.10
Random intercept variance	1.41			0.00		
Residual variance	2.52			7.89		

Table 2-2. Linear mixed effects model for combined datasets from April 2012 and April 2013 with year as a factor variable and an interactive effect of burn treatment and year.

Combined Model for April 2012 and April 2013 w/ Year as Factor			
<i>Parameter</i>	<i>Estimate</i> ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	<i>DF</i>	<i>P</i> <i>value</i>
Fixed intercept (β_0)	4.55±0.45	175	<0.001***
+Burn treatment	1.96±0.51	175	<0.001***
+Nitrogen treatment	1.10±0.33	175	<0.01**
+CO₂ treatment	1.02±0.37	175	<0.01**
Year (2013)	0.47±0.59	175	>0.3
+Burn*Year (2013)	-2.16±0.72	175	<0.01**
Random intercept variance	0.96		
Residual variance	2.39		

Structural Equation Modeling

Consistent with observed effects of global change treatment factors on environmental conditions and plant productivity, structural equation modeling indicated that, in the first growing season following fire, soil CO₂ efflux rate was directly increased by the fire, N deposition, and elevated CO₂. It was also increased by higher surface soil temperature, fine root biomass, tap-root biomass in the top 15cm of soil, and total aboveground plant biomass.

Relevant indirect effects included the positive influence of fire on soil temperature, positive effects of fire and elevated CO₂ on fine root biomass, and positive influence of fire and N deposition on total aboveground biomass. While elevated precipitation had a direct positive influence on soil CO₂ efflux, taproot biomass was negatively influenced by increased precipitation, which itself was a positive, direct influence on soil CO₂ efflux. Thus, while direct comparisons of soil CO₂ efflux rates in elevated precipitation plots showed no differences from ambient precipitation plots, structural equation modeling revealed that this was due to counter-acting direct and indirect effects (Fig 2b). The final path analysis model was determined by removing all non-significant path coefficients from the *a priori* model that included all pathways between variables. Soil moisture was absent from the final model, even though it was strongly influenced by both the global change treatments and the fire and was weakly correlated with soil CO₂ efflux.

The final model, shown in Fig. 2b, is a good fit for the observations ($X^2=17.246$, d.f.=16, $p=0.370$, indicating no difference between model and observations, Goodness of Fit Index=0.999, Tucker Lewis Index=0.984, Comparative

Fit Index=0.992, Root Mean Square Error of Approximation= 0.03, Standardized Root Mean Residuals=0.046, all path coefficients were significant at the $p < 0.10$ level.) The path diagram has an R^2 value of 0.47, indicating that, taken collectively, the direct and indirect effects of the global change treatments, mediated by soil temperature and fine root biomass, explain 47% of the variance of soil CO₂ efflux rates in 2012, a reasonably high explanatory power for ecological measurements in an uncontrolled field setting (Siqueira *et al.* 2015). In temperate forests seasonal soil temperature variation has been found to explain 80% of the variance in soil CO₂ efflux (e.g. Davidson *et al.* 1998).

Simulated Season-Initiating Rainfall Experiment

In late July 2013, a simulated 20mm rainfall event immediately increased soil CO₂ efflux in both +N and ambient N plots by an average of more than six-fold, relative to plots which received no wetting treatment. In all cases, CO₂ efflux rapidly increased following wet-up and then gradually diminished, returning to pre-wet-up levels by 24 hours post-wet-up (Fig. 4). An average of 13.9 ± 2.3 g CO₂ m⁻² was released from soils as a result of the simulated precipitation event over the course of 48 hours, compared with 4.0 ± 4.9 g CO₂ m⁻² from plots that did not receive the wet-up treatment. This pulse of ~ 10 g CO₂ m⁻² over 48 hours represents just under 1% of total annual above- and belowground NPP in this ecosystem (assuming the 2012 average 576g m⁻² total above- and below-ground biomass and 50% of that weight as C).

In contrast with measurements made during the peak growing season in both April 2012 and April 2013, there was difference between +N and ambient-N plots in

either peak soil CO₂ efflux rate or in total excess CO₂ above control released over 48 hours ($p>0.15$). There was also no effect of the +Burn treatment, nor any other global-change treatment factor on the magnitude of the soil CO₂ pulse released by season initiating wet-up. Simulated summer rainfall increased soil moisture content in the top 5cm by an average of 13% relative to non-wetted soil. Soil moisture levels and soil CO₂ efflux rates returned to pre-treatment levels by 48 hours after wet-up.

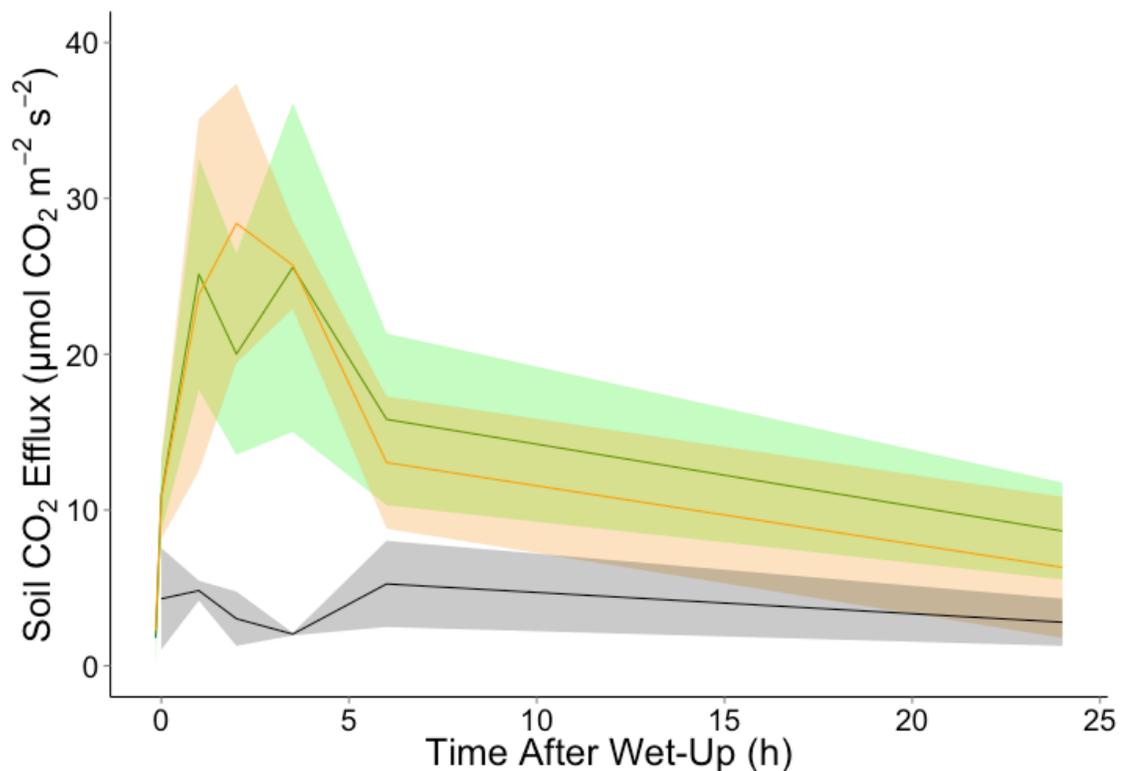


Fig. 2-4. Soil CO₂ efflux ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) over 24 hours in response to a simulated 20mm rain event at $t=0$ is shown for +N plots in green and ambient N plots in orange. Black indicates un-wetted control plots. Standard deviations are shown as colored bands around averages of 6 replicates, with three measurements made at each replicate at each time point.

DISCUSSION

Effects of Global Change Treatments on Soil CO₂ Efflux

After a decade and a half of warming, warmed plots did not exhibit greater rates of soil CO₂ efflux than ambient temperature plots during the growing season. These data probably demonstrate an acclimation of soil CO₂ efflux to moderate warming, though it is possible that CO₂ efflux at other times of the year is increased by warming.

Long-term N addition increased soil CO₂ efflux during April of 2012 and 2013. Structural equation modeling suggests that the effect of +N on soil CO₂ efflux is due to a greater supply of labile substrate associated with increased aboveground production. N addition increased total aboveground biomass (Bonferroni adjusted $p < 0.02$) by an average of 140 g m⁻² in April 2012, and total aboveground biomass was strongly correlated with soil CO₂ efflux. The effects on total aboveground biomass were driven through an increase in the biomass of annual forb species, in particular. Fine root production was marginally lower (though not significantly) in plots with added N compared to plots without.

Our results are consistent with those of Craine and colleagues (2001), who found an 8% increase in soil CO₂ efflux due to N fertilization in a grassland ecosystem, corresponding to a significant increase in aboveground biomass, with no significant change in fine root production. Similarly, Dijkstra and colleagues (2005) in the same experimental set up, found that N fertilization increased aboveground biomass and increased CO₂ efflux rates, despite reductions in labile C and root exudates. They concluded that an increase in litter quality and biomass accumulation mediated the effect of N fertilization on soil CO₂ efflux (Dijkstra *et al.* 2005). The similar positive effects of +N enrichment on soil CO₂ efflux over the two measured

years, mediated by increased aboveground production, suggest that this is a consistent effect in this grassland ecosystem, though the effects of experimental N deposition on ecosystem biogeochemical responses in grassland are non-linear (Gomez-Cassanovas *et al.* 2015). In contrast, Janssens and colleagues, in a large meta-analysis of temperate forest ecosystems, found that anthropogenic N deposition reduces organic matter decomposition and decreases soil CO₂ efflux rates (Janssens *et al.* 2010).

Elevated CO₂ also marginally increased soil CO₂ efflux rates across both years (combined multi-year mixed effects model $p < 0.01$, separate year models: $p < 0.20$). Consistent with the results from previous grassland studies, this effect was strongly mediated by increases in fine root production in elevated CO₂ plots (Hungate *et al.* 1997; Craine *et al.* 2001; Dijkstra *et al.* 2005).

The responses of soil CO₂ efflux to long-duration (15 year) global change are consistent with durable acclimation of soil respiration to moderate soil warming, as well as a response to N addition and elevated CO₂, where added N stimulates the accumulation of aboveground biomass, increasing soil CO₂ efflux rates from these extra C inputs (even when root production does not increase), while atmospheric CO₂ enrichment increases root production and root exudates leading to greater soil CO₂ efflux. The net result is that soil CO₂ efflux is strongly mediated by NPP increases; for +N enrichment the pathway occurs through impacts on aboveground production, whereas for +CO₂ enrichment, the pathway occurs through impacts on fine root production. Importantly, neither of these pathways suggests a change in the size of the soil C pool; rather the effect of the global change treatments is to accelerate the C cycle.

Short-Duration Treatments: Effects of Fire

Low-intensity fire increased CO₂ efflux from grassland soils at the time of peak-biomass. These effects were short-lived, occurring only during the first growing season after the experimental fire. Based on a combination of structural equation modeling and contrasts with data from the following growing season, this effect can be attributed to three mechanisms: the fire's effects on surface soil temperatures (+), fine root biomass in the top 15cm of soil (+), and aboveground NPP (+).

First, soils in plots subjected to fire treatment were warmer than soils in control plots by 5°C at the time of measurement and nearly 3°C during the two-week interval containing the measurements. Soil temperature is a strong controller of short-term soil CO₂ efflux rates in many ecosystems. In our study, soil temperature was positively correlated with soil CO₂ efflux, and burned plot soils were warmer than unburned plot soils. The warming effect of fire in the prior summer was substantially larger than that of the long-duration warming treatment. Thus, while grassland soils had acclimated to long-duration, decadal-scale warming of 1-2°C, the “extra” soil warming from the controlled burn increased rates of soil CO₂ efflux.

The elevated soil surface temperature in burned plots was a consequence of the removal of litter by the fire. Soil surface temperatures were negatively correlated with standing litter biomass (simple linear regression: $F=18.63$, $d.f.=121$, $p<0.01$), which was depleted 10-fold due to the fire. These results – both the magnitude of soil warming from the burn and litter removal – are similar to the effects observed by Henry et al. (2006) after the 2003 fire in the Jasper Ridge Global Change Experiment.

In a separate study in a grassland in Kansas, Blair (1997) found a 6°C mean daily soil temperature increase in the growing season following a burn, also due to litter removal.

Second, the fire increased total fine root production (Bonferroni adjusted $p < 0.01$), which was strongly correlated with soil CO₂ efflux rates. Increases in fine root production and root exudates – which represent a source of labile C substrate – increase soil CO₂ efflux rates in grassland ecosystems (Reich *et al.* 2001).

In addition to fine root production, the fire treatment increased total aboveground biomass (Bonferroni adjusted $p < 0.01$) in the subsequent growing season by an average of 174.5g m⁻² in 2012, and greater aboveground biomass was strongly correlated with greater soil CO₂ efflux (Figure 2B). Fire likely increased NPP through a combination of a reduced early season shading by litter removal (Henry *et al.* 2006) and potentially through a fertilization effect, where litter combustion increased N and P inputs (Henry *et al.* 2006, Augustine *et al.* 2014, Wang *et al.* 2014).

Structural equation modeling suggests that the effect of increased NPP on soil CO₂ efflux proceeds via both above- and below-ground pathways. While aboveground and belowground biomass production were weakly correlated with each other, fire increased both, and both were positively correlated with soil CO₂ efflux, even when controlling for the effect of the other. Path analysis that was restricted to measurements from grassland plots that were burned and also received no N addition revealed strong effects of soil temperature, aboveground biomass, and fine root biomass, though temperature and fine root biomass had larger standardized coefficients. The relative magnitude of these last two effects is very similar (as shown

in the standardized parameter values in Fig. 2b). The fact that the effect of the fire lasted only a single growing season also suggests that fine root production and decomposition of exudates and temperature effects from litter removal, rather than NPP-driven changes in soil organic matter, play strong roles on driving increased soil CO₂ efflux after fire.

Our results are consistent with those of Knapp and colleagues (1998) who studied the response to fire in tall-grass prairie in Kansas. In that system, annual fire increased respiration rates by around 33% due to a combination of increased soil temperatures (4-6°C) and belowground activity (Knapp *et al.* 1998), with only moderate effects of soil moisture. Xu and Wan (2008) also found increases in soil CO₂ efflux following fire in a grassland in China, though these increases were associated with increases in soil moisture rather than changes in soil temperature. Most recently, Muñoz-Rojas and colleagues (2016) also found increases in respiration in response to fire in a semi-arid grassland due to increases in soil temperature (Muñoz-Rojas *et al.* 2016).

All of our measurements of soil CO₂ efflux rates were made from a bare-soil substrate with litter removed. This measurement approach may explain the differences between our results and those of Niboyet and colleagues (2011a), who included CO₂ efflux from litter in their measurements and who found no significant increases in CO₂ loss from soils in the year after an accidental fire. Removing standing litter from soil collars likely reduced measured soil CO₂ efflux rates from unburned plots in our study. In comparing burned and unburned plots, our estimates do not address the contribution to ecosystem respiration from litter decomposition in the

unburned plots. However, the magnitude of respiration fluxes from standing litter in unburned plots would have been less than or equal to the amount of C released from the combustion of litter during the fire itself. The experimental fire released approximately 100g C m^{-2} of from the ecosystem. Thus, our measurements of increased soil CO_2 efflux rates from bare soils following fire represent additional soil C loss from burned plots relative to unburned plots.

Short Duration Treatments: Effects of Season-Initiating Wet-Up

During the dry season, soil moisture is the dominant control on soil C cycling. The mechanism (growing season NPP) underlying the positive effects of the +N treatment on soil CO_2 efflux is limited to the growing season when the plant and microbial communities are metabolically active. Specifically, the effect of elevated aboveground NPP increasing soil CO_2 efflux does not carry over to the subsequent season-initiating wet-up, suggesting that it is more due to root respiration and decomposition of exudates rather than increased soil organic matter resulting from increased NPP.

Our simulated rainfall event can be understood as a proxy for the response of soil C dynamics to natural wet-up at the beginning of the wet season. The first rainfall event of the season in dry climates can have profound effects on CO_2 cycling (e.g. Bowling *et al.* 2011). Our results indicate that, while respiration increases dramatically during the first wet-up of the season, wetted soils released a pulse of only 0.9% of annual total NPP. The simulated wet-up occurred about one third of the way through the dry season, suggesting that the pulse with the natural onset of the rainy season

could be several-fold larger but still a small fraction of annual NPP. This contrasts with the pattern in other systems (Borken and Matzner, 2009), perhaps because the CO₂ pulse was cut short by rapid drying, or because of continuous decomposition during the dry season, so that highly labile materials do not accumulate.

Ecosystem C Effects

How CO₂ losses respond to the combination of anthropogenic global change and punctuated disturbance (which may themselves be altered by global change) determines the aggregate feedback of anthropogenic global change to the C cycle. Decadal-scale soil surface warming of 1-2°C did not increase soil CO₂ efflux rates at the time of peak biomass. At this time, however, *additional* warming of 3-5°C associated with a low-intensity fire the previous summer increased soil CO₂ efflux rates.

Our results also add to growing and consistent evidence from multiple grassland ecosystems that fire increases growing-season soil CO₂ efflux through some combination of abiotic effects on temperature (and soil moisture) and biotic effects on belowground production (Knapp *et al.* 1998; Xu and Wan, 2008). Thus, fire regimes and fire disturbance should be taken into account when assessing grassland C budgets.

In this ecosystem, fire induced a significant increase in both above- and belowground NPP, and a roughly proportional greater efflux of CO₂ from soils the following growing season. This result suggests that, rather than increasing the net CO₂ uptake of the ecosystem, or increasing the net C loss from soils over time, fire may

effectively increase the rate of C cycling during the subsequent growing season, with more CO₂ fixed, but more CO₂ lost from soils from root respiration.

Implications for C accounting

Our results demonstrate the effects that short-duration punctuated events—a low-intensity burn and season initiating rainfall—can have on C cycling in a grassland ecosystem. For accurate accounting, fluxes should be resolved on temporal and spatial scales sufficient to account for this kind of short-duration punctuated event.

Chapter 3

Barriers to incorporating climate change mitigation ecosystem services into coastal conservation practice: the case of blue carbon³

ABSTRACT

Non-governmental organizations, academic scientists, and regulatory agencies in the United States are rapidly developing new institutional environmental governance frameworks around the concept of ecosystem services. This construction is taking place in the context of rising federal government interest in—and, indeed, insistence on—the use of ecosystem services in decision-making. As such, ecosystem service valuation as a basis for decision-making is quickly becoming a dominant paradigm within environmental management. Although valuation of ecosystem services has received substantial attention in the literature, relatively little is understood about these new forms of ecosystem service governance. Using the case of coastal blue carbon, we assess barriers to—and opportunities for—the uptake of the ecosystem service concept among stakeholders in these services: local, place-based coastal conservation organizations. Based on semi-structured interviews with individuals working in such coastal conservation organizations in two U.S. regions, our data suggest a typology of five barriers to action on blue carbon: (1) time, financial, and resource barriers; (2) expertise and/or technical barriers; (3) political barriers; (4) motivation/identity barriers; and (5) localism barriers. Our results also

³ A version of this chapter, written with Nicole M. Ardoin, is currently under revision at *Ecology and Society*.

suggest two necessary conditions for action on blue carbon within a local organization: a connection to a research laboratory and an awareness of—or connection to—the national nonprofit conservation organization Restore America’s Estuaries. Taken as a whole, our work makes clear that local, place-based coastal conservation organizations in the United States have not yet mainstreamed the concept of ecosystem services. As the ecosystem services concept enters a broad swathe of federal environmental decision-making, careful attention to its reception, perception, dissemination, and evolution at local scales is essential if the institutional development is to be successful and sustained.

INTRODUCTION

Through rapid and persistent anthropogenic global change, we now recognize that human activity is at risk of fundamentally altering the functioning of the earth system (Rockström et al. 2009). The consequences for human livelihoods of such changes are likely to be devastating (Steffen et al. 2015). As humanity has entered the Anthropocene (Crutzen 2006) over the previous two decades, we have witnessed growing calls for an increased recognition of ecosystem services—the values that natural ecosystems provide to humans—and for actions to protect those values (Daily et al. 2009).

Numerous recent studies have highlighted how considering ecosystem services can inform environmental management by internalizing costs of environmental damage into markets (Gómez-Baggethun et al. 2010), elucidating tradeoffs (Goldstein et al. 2012; Lester et al. 2013), and highlighting the potential for win-win solutions

(Howe et al. 2014). Although much of the work around ecosystem services has been produced by academic scientists and non-governmental organizations (NGOs) focused on ecological conservation (Peterson et al. 2010), recently there has been increasing consideration of ecosystem services within formal environmental governance contexts. New institutional frameworks—from models built to include ecosystem services used by coastal planners, to regulations that outline programs for payments for ecosystem services, to nutrient pollution trading markets—are being constructed around the concept of ecosystem services. This construction is taking place in the context of rising federal government interest—and, indeed, insistence—in the use of ecosystem services in decision-making (Donovan et al. 2015). NGOs, academic scientists, and regulatory agencies have recently developed new guidelines and frameworks for how to consider ecosystem services in decision-making (Rosenthal et al. 2015; Olander et al. 2015). Ecosystem service valuation as a basis for decision-making is rapidly becoming a dominant paradigm within environmental management; relatedly, outlining approaches that describe how to consider of ecosystem services within existing management frameworks is a rapidly growing enterprise (Schaefer et al. 2015).

Managing ecosystem service resources

In light of this increased attention, it is important for environmental governance scholars to understand both the institutional, and also the broader social-ecological, context of ecosystem services. Although the concept and valuation of ecosystem services have received substantial attention in the literature, relatively little

is understood about ecosystem service governance (Primmer et al. 2015). Below, we highlight four challenges that emerge from the literature describing the current understanding of nascent ecosystem service governance institutions, including: (1) the unclear nature of the resource, (2) mismatches of scale, (3) top-down institutional frameworks, and (4) unclear stakeholder identification. We address each of these challenges in detail in the following sections.

Unclear nature of resources

Ecosystem services are, in effect, a form of natural resources (Costanza et al. 2014). Natural resources are frequently categorized as falling into one of four categories (see Figure 3-1A): private goods, club goods, common-pool resources, and public goods, and are based on the ease of excludability of resource users from the resource and whether the consumption of the resource is rival (i.e., the use of the resource by one user diminishes the use of the resource by another user). Different ecosystem services fall into these four resource categories. Ecosystem services themselves, traditionally, are also divided into four categories (e.g., Liu et al. 2015): provisioning, supporting, regulating, and cultural services (Figure 3-1B). Although many ecosystem services are considered common-pool resources, many regulating and supporting ecosystem services associated with provisioning a stable climate or maintaining water quality are traditionally thought of as public goods, the use of which is non-exclusive and available to (Muradian and Rival 2012; Costanza et al. 2014).

Categorization of Natural Resources	Rival in Consumption	Non-rival in Consumption
High Excludability	Private Goods	Club Goods
Low Excludability	Common-Pool Resources	Public Goods

Figure 3-1A. Typologies of environmental resources. Private goods are those that are rival in consumption and have high ease of excludability. Club goods are those that are not rival in consumption, but have high ease of excludability. Common-pool resources are those that are rival in consumption, but have low ease of excludability. Public goods are those that are not rival in consumption and have low ease of excludability.

ECOSYSTEM SERVICES

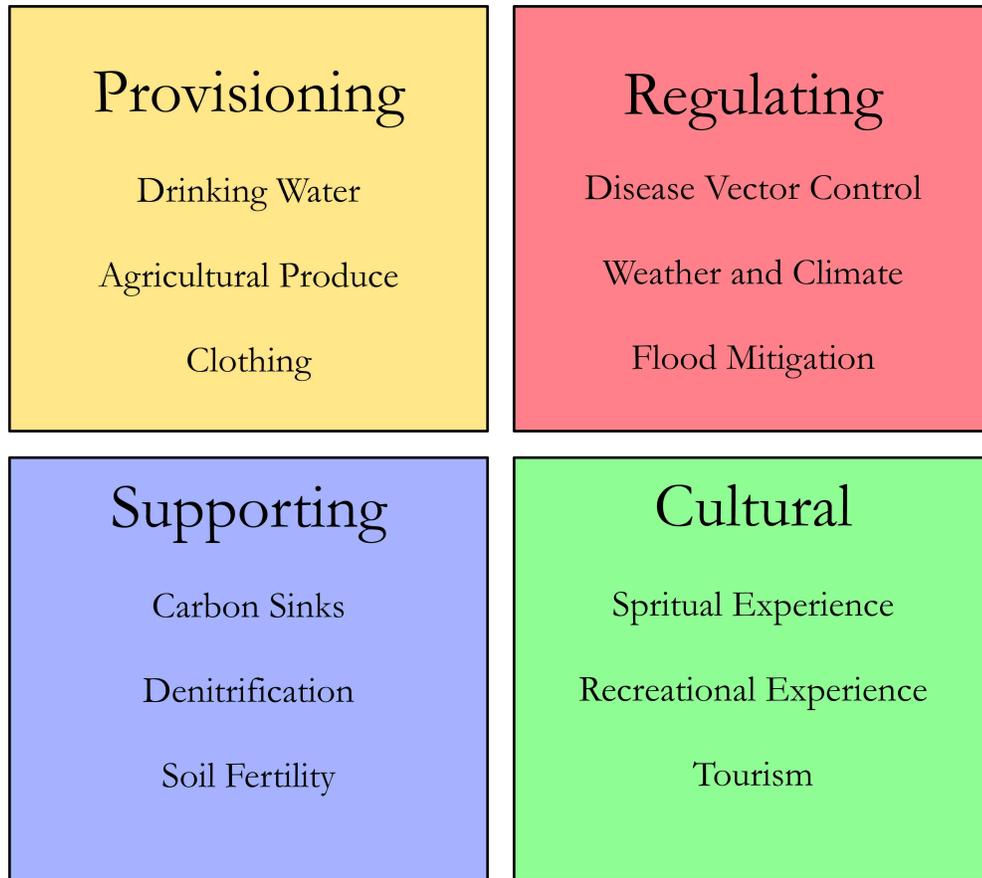


Figure 3-1B. Typology of ecosystem services

Much of the theoretical understanding of natural resource management is built around an understanding of what kind of good a particular natural resource represents. In particular, privatization of common-pool resources and public goods is a widely suggested, yet problematic and heavily criticized, governance strategy for conservation of resources to overcome the perception of the tragedy of the commons (Hardin 1968). Public goods have frequently proven challenging to privatize in practice, given the difficulty of making them both excludable and rival in consumption (Costanza et al. 2014) and the information and uncertainty challenges associated with allocation (Thompson 2000). Yet, programs including payments for ecosystem services (PES), especially market-based instruments (MBI) built around climate mitigation services that seek to preserve a resource (such as carbon sequestration), attempt to privatize that which is often considered to be a public good.

Furthermore, in light of the increasing recognition of the finite boundaries and limits to ecosystem functioning, numerous workers have begun to define the act of greenhouse gas emissions as ‘consuming part of a finite atmospheric carbon budget,’ in effect (though perhaps not intent) recasting atmospheric carbon dioxide concentrations as a common pool resource (rival in consumption) rather than a public good (Kanitkar et al. 2010; Edenhofer et al. 2013). As such, the resource identity of different ecosystem services is mutable and evolving, creating challenges for understanding the institutional and social-ecological contexts of ecosystem service governance.

Mismatch of scale

Many public goods and common-pool resources are managed at a range of geographic, social, and jurisdictional scales. Some ecosystem services and benefits—such as water-quality maintenance, flood mitigation, recreational opportunities, and local cooling effects—are delivered at local scales to resource stakeholders who live and work locally (Fisher et al. 2009). Other ecosystem services and benefits, such as carbon sequestration, are delivered at global scales and in more diffuse ways (Hein et al. 2006). Management actions that generate and promote these services, however, may be disconnected spatially and temporally from the realization benefit. Single actions, taken locally, can also generate benefits across scales. Local-scale decisions about wetland restoration, for example, may simultaneously provide local-scale benefits and also globally diffuse benefits. The mismatch of scale of ecosystem service governance, provisioning, and benefits can exacerbate the challenges of ecosystem and resource management (Gómez-Baggethun et al. 2013).

Top-down dissemination

Further complicating the governance of ecosystem services at local scales is the top-down manner in which notions of ecosystem-service management have been disseminated. The ecosystem service concept initially emerged from academic scientific researchers. After a push for moving the concept into the mainstream conservation discussion (Young 2013), conservation NGOs, as well as federal agencies, have subsequently adopted, and perhaps transmuted, the concept (Donovan et al. 2015) and framing of ecosystem services; carbon governance, in particular, is

also frequently linked with the forces of globalization (Ernstson and Sörlin 2013; Stripple and Bulkeley 2013; Backstrand 2004). The top-down structuring of ecosystem service management institutions, including those that involve PES, creates a tension with much of the theoretical and empirical understanding of what makes common-pool resource management institutions function and endure, especially at local scales (Ostrom 1990; Cox et al. 2010). The current institutional design of ecosystem service management systems is not one that matches the empirical experience for successful common-pool resource management institutions.

Stakeholder identification

Perhaps what most complicates the understanding of these new institutional mechanisms for managing ecosystem services is the identification of stakeholders in ecosystem services resource provisioning and delivery. In part, the challenge posed by ecosystem service resource management, unlike most common-pool resources, is the livelihood of those involved in provisioning ecosystem services do not generally rely upon the resource (Acheson et al. 2006). Only under the most extreme circumstances are those involved in conserving wetlands, for example, risking their livelihood with their decisions; by contrast, the livelihood of foresters and fishers are inextricably linked with the fate of those resources. In the current theoretical conceptualizations of resource management within social-ecological contexts, local stakeholders are essential to functioning resource management systems (Ostrom 2009). The lack of clarity over who has a stake in ecosystem service resource management complicates our understanding of these nascent governance contexts.

Carbon sequestration management

All four of the challenges described above are particularly pronounced when investigating the rise of carbon sequestration management institutions; thus, this chapter is organized around these services. Although carbon sequestration would appear to be a public good, many nascent institutional approaches seek to cast climate mitigation resources as rival in consumption. In particular, ecological carbon sequestration is increasingly commodified within voluntary and compliance-carbon markets operating at international, regional, state, and local scales (CARB 2015). Unlike many other ecosystem services, a distinct mismatching of scales occurs between local actions required to provide carbon sequestration ecosystem services and the globally diffuse benefits they provide. The service provided by carbon sequestration is the marginal effect on overall climate mitigation—the reduction in the social cost of climate change—a benefit realized by each individual on the planet. Understanding how place-based, local conservation organizations, as potential stakeholders in carbon cycle ecosystem services, are engaging with this new top-down resource management framework is critical, not only to our theoretical understanding of public good and common-pool resource management, but also to assessing the future shape of conservation policy and practice.

To assess the role(s) that local conservation organizations play as stakeholders in managing climate mitigation ecosystem service resources, we analyze the case of the management of coastal ecosystem carbon sequestration—blue carbon—by local, place-based coastal conservation organizations in two U.S. regions.

BACKGROUND

The case of blue carbon

Before presenting our analysis, we first describe what constitutes blue carbon, as well as the political and policy contexts in which this interest in blue carbon has grown. Any ecosystem where net primary production (from photosynthesis) is greater than respiration over time functions as a natural carbon sink in that the flux of carbon from the atmosphere into the ecosystem is greater than the loss of carbon from the ecosystem. Over time in ecosystems that are carbon sinks, carbon accumulates in soils, organic matter, peat and/or standing vegetation, such as trees. If the carbon that has accumulated remains out of the atmosphere for a long time—generally understood to be at least 100 years in ecosystem governance—it is said to be ‘sequestered.’ The carbon sequestered and stored in coastal ecosystems, is known as blue carbon, and has been highlighted increasingly as a meaningful and policy-relevant ecosystem service (Ullman et al. 2013).

Salt marshes, mangroves, sea grass meadows, and tidal flats are all carbon sinks, as they fix carbon not only from the atmosphere, but also trap and accumulate organic carbon delivered from outside the ecosystem by tides, waves, currents, and rivers. Globally, these blue carbon ecosystems sequester on the order of hundreds of millions of metric tons of CO₂ each year (McLeod et al. 2011), nearly equivalent to the emissions from the economy of the U.S. state of California. On a per-unit-area basis, blue carbon ecosystems are among the largest carbon sinks on the planet (Chmura et al. 2003; McLeod et al. 2011).

In many regions of the world, blue carbon ecosystems are rapidly being degraded or destroyed for aquaculture, such as shrimp farming in Southeast Asia or urban development along coastlines (Pendleton et al. 2012). In the United States, many blue carbon ecosystems have already been destroyed. In California, over 90% of historical coastal wetlands have been destroyed (California Coastal Commission 2013), and in coastal New England, 37% of the original, pre-1800 salt marshes have been lost, with over 80% loss in areas around Boston (Bromberg and Bertness 2005). Environmental nonprofit organizations, academic scientists, and government agencies have indicated growing interest in building institutional mechanisms to manage the service of carbon storage within coastal ecosystems (Hejnowicz et al. 2015; Nellemann and Corcoran 2009). Agency-affiliated researchers have issued calls to incorporate coastal carbon sequestration services into existing statutory and regulatory frameworks in the United States, highlighting that new legislation is not needed to build institutional frameworks based on ecosystem services (Sutton-Grier et al. 2014).

Unlike forest carbon, blue carbon is not currently included within California's cap-and-trade program's menu of carbon offsets; however, Restore America's Estuaries (RAE), in conjunction with partner group Silvestrum, has recently developed a set of rules concerning the accounting for blue carbon services for use in the non-regulatory-compliance, voluntary market (RAE 2015). RAE, a national non-profit organization formed in the 1990s, has led U.S. efforts to advocate for enhancement and management of blue carbon ecosystem services, sponsoring several major pilot blue carbon projects, attending national scientific conferences to present information about blue carbon research, and convening workshops on blue carbon.

We, thus, situate our analysis of place-based coastal conservation organizations that steward coastal ecosystems at local spatial scales in this context. We examine these organizations in light of the growing interest in incorporating blue carbon ecosystem services into decision-making and promoting mutually beneficial coastal habitat restoration, as well as coastal carbon sequestration.

METHODS

Coastal conservation organization identification

We seek to understand the role of local coastal conservation organizations as stakeholders in the management of ecosystem service resources. Our research is particularly concerned with how locally based organizations—as stewards of particular geographies—interact with a management paradigm that has been developed, promoted, and reproduced by a set of international NGO, academic, and federal agency actors. As such, our sampling population was composed of non-profit coastal conservation organizations with organizational missions dedicated to the conservation of a particular coastal geography, often a single estuary, lagoon, coastal watershed, or embayment. Any national and international-scope organizations, as well as local organizations that were not focused on the conservation of a specific coastal geographic location, were excluded from analysis. Sampling was agnostic to the property-ownership status of place-based coastal conservation organizations. Some organizations were property owners; for example, coastal or estuarine land trusts. Other organizations were stewards of lands or aquatic spaces owned by the state or other private property owners, often focused on water quality maintenance. The

majority of organizations were chartered 501(c)3 nonprofit organizations in the United States.

To increase the likelihood of including within our sampling population some organizations that are actively engaging in the management of carbon sequestration ecosystem service resources, we restricted our study to place-based coastal conservation organizations in the California Current and Gulf of Maine bioregions (Figure 3-2). California and the New England states are also among the few sub-national jurisdictions in the United States that have active climate change policies. Both of these regions also have active and robust traditions of coastal conservation that have been linked with concerns related to climate change (Heberle et al. 2014; Chornesky et al. 2015). We sampled in these regions as they were more likely to include organizations that were innovative in terms of incorporating emergent issues and frames, such as ecosystem services, into their work (Ruckelshaus et al. 2013; Börger et al. 2014).

We combined extensive online searches and snowball sampling to identify a list of 71 potential place-based coastal conservation organizations within these two regions that met our criteria. Organizational participation was solicited via an email sent to an executive director, conservation director, education/outreach specialist, or general contact email address at each identified organization. Organizational participation in this study was anonymous (See Figure 3-3 for an overview of the methods used.)

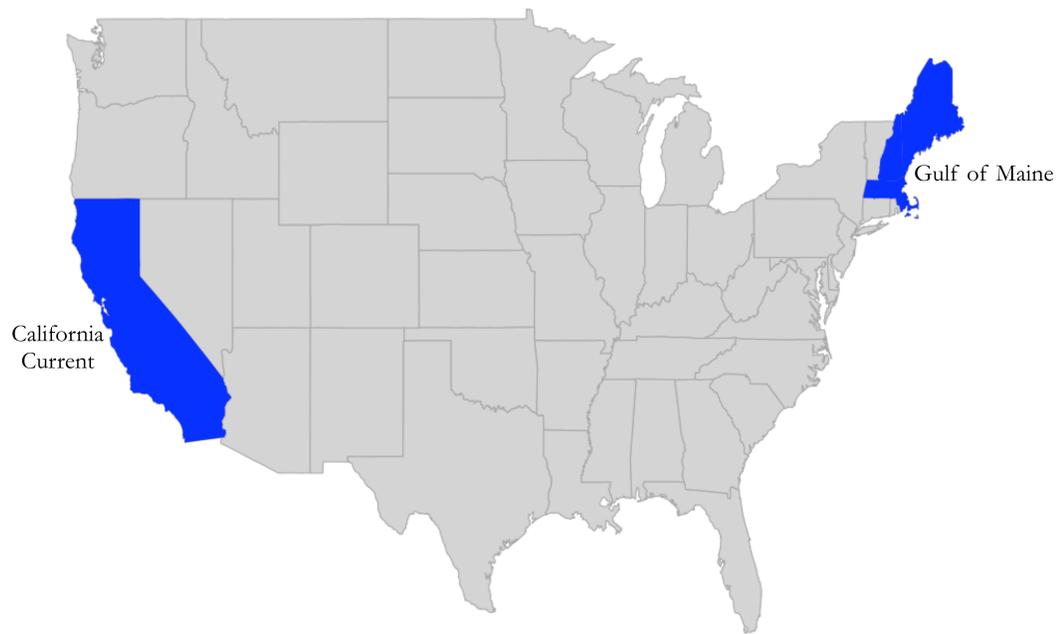


Figure 3-2. Map of United States showing study areas in blue.

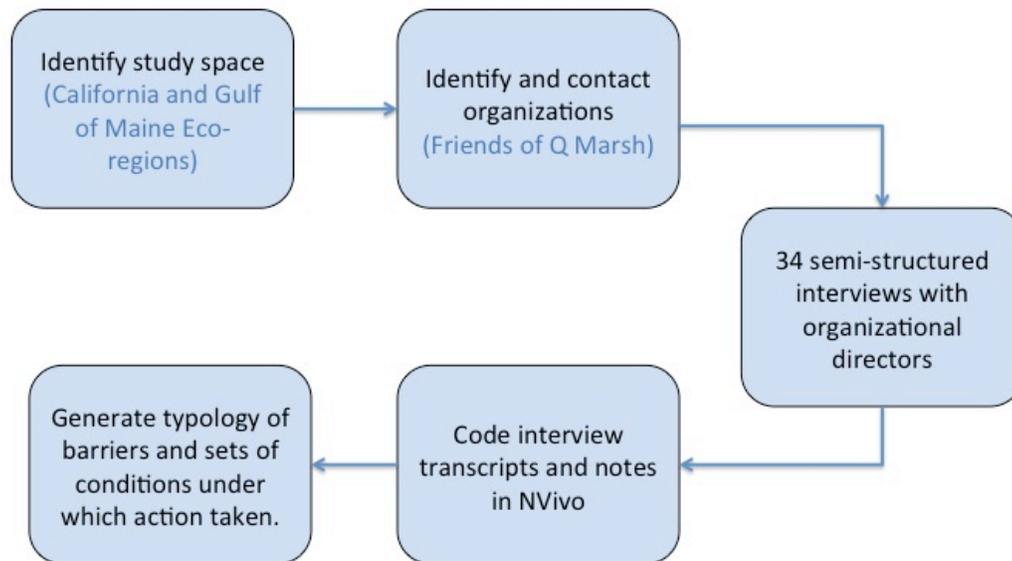


Figure 3-3. Conceptual flow-path diagram of methodological approach.

Semi-structured interviews: coding and analysis

We conducted 34 semi-structured interviews with representatives of coastal conservation organizations between June 2012 and December 2014. Interviews were conducted either in person or via telephone. When possible, the interviews were recorded for later transcription; if recording was infeasible or not acceptable to interviewees, extensive notes were taken. Interviews focused on organizational engagement with ecosystem services, generally, and blue carbon services, particularly organizational missions and individual perceptions of barriers and facilitators related to the ecosystem services or blue carbon concept. Interviews lasted between 30 minutes and three hours.

Interview transcripts and/or notes were coded using NVivo Qualitative Analysis Software (QSR International). Coding focused on three primary areas: defining action on blue carbon, identifying barriers to action, and identifying conditions under which action was taken. We describe each area of coding emphasis below.

First, we categorized organizations dichotomously as either engaging in ‘action’ on blue carbon or not engaging in action. To do so, action on blue carbon was defined as one of the following: (1) any project or other activity undertaken by the organization designed, in whole or in part, to enhance carbon sequestration of the coastal ecosystems being managed or stewarded by the organization; (2) any written or publicized statement highlighting or mentioning the carbon sequestration or climate change mitigation value, whether qualitative or quantitative, of the coastal ecosystem being conserved by the organization. Second, among organizations engaging in action, we coded actions based on whether blue carbon or carbon sequestration ecosystem services were mentioned in materials, a defined project to enhance carbon-sequestration ecosystem services was being conducted, and ecosystem services were being quantified.

We then used an iterative, modified grounded theory approach (Corbin and Strauss 1990) to develop a typology of barriers to the incorporation of ecosystem services into the work of coastal conservation organizations. First, we coded ‘barriers’ based on the content of interview transcripts, where any reason given for a lack of action on, or interest in, carbon ecosystem services was identified as a barrier. Using this first set of barriers as a base, we then identified a second set of emergent

categories of barriers. After reviewing literature on barriers to conservation action and organizational behavior, we subsequently re-coded the second set of categorized barriers to align with literature-derived categories.

For those organizations coded as having taken (or as planning to take) some form of action on blue carbon, we coded for enabling conditions within interview notes and transcripts; those from organizations categorized as ‘not engaging in action’ were then reviewed for the presence or absence of each of these same categorized enabling conditions.

RESULTS

Minimal action on blue carbon

Although all of the place-based local coastal conservation organizations in our sample are engaged in some form of coastal ecosystem restoration or habitat conservation projects that might be understood to include enhancing coastal carbon sequestration through a simple description that would not require additional resources, the majority (82%) of these organizations reported that they are not currently taking any action on blue carbon, nor do their conservation missions include enhancing climate change mitigation ecosystem services. The organizations that reported taking some kind of action on carbon cycle ecosystem services (18% of those contacted) were primarily engaged in pilot projects focused on enhancing living shorelines, quantifying blue carbon storage in conjunction with other ecosystem services, or otherwise focused on ameliorating hypoxic conditions with explicit connections to carbon cycling.

Typology of barriers

Based on the data, we developed a typology of five distinguishable barriers to action on carbon cycle ecosystem services: (1) time, financial, and resource barriers; (2) expertise and/or technical barriers, (3) political barriers, (4) motivation/identity barriers, and (5) localism barriers. Below, we consider each of these in more detail, supported by examples from the data.

Temporal, financial, and resource barriers

Numerous respondents indicated that they lacked the financial resources to pursue a blue carbon project or, otherwise, had too many projects already in process. In these circumstances, the interviewee was sympathetic to the desire to engage in work oriented toward the ecosystem services frame (although they were not hesitant about such work), but felt that other needs were more pressing and, therefore, that the organization's limited resources—in terms of employee and volunteer time, as well as financial resources—were prioritized in such a way so as not to allow “taking on something new.” One employee described it as follows:

We're barely able to do what we've already got going on, so we haven't really thought about it ... [we haven't] had time to think about it ... to be honest.

The Executive Director of another coastal watershed alliance noted that the volunteer nature and large number of issues involved in conservation work could be prohibitive to engaging with carbon cycle management:

It's not that I don't want to do these kinda things ... it's just ... I mean I'm a volunteer, you understand, there's no salary involved ... and I mean we're involved in this whole litigation thing ...

Expertise and/or technical barriers

Several conservation directors indicated that they had contemplated trying to engage in a carbon-cycle-oriented pilot project, but described becoming quickly overwhelmed by the technical details and requirements for measuring and monitoring. The director of one organization indicated that they started work in this vein, but realized that the measurements they could make (mostly from measuring soil and peat samples) would not be sufficient for quantifying carbon dioxide (CO₂) gas fluxes; therefore, in the end, they pursued a different tack:

We talked about doing that, but we didn't have any of the expertise and the more we read, it was like ... we didn't know where to begin.

As one Executive Director of a non-profit organization highlighted,

We decided to do a study ... but we didn't have any intention of looking at the gas fluxes, I mean, I was like, "Are you kidding me with this stuff?" So maybe the complexity of the gas fluxes are, y'know, along the lines of why no one's really trying to do [blue carbon] ... it seems.

For other organizations, the challenge related to connecting the work to something abstract, such as carbon. Whereas the organizations did focus on the values of the services provided by ecosystems, when it came to the carbon and nitrogen cycling, they backed away from specifics. For some organizations, the lack of availability of adequate information was a major strategic need to be filled before such actions around ecosystem services could be contemplated. As one respondent said,

The biggest challenge I think ... I would say it's the lack of [biogeochemical] data, actually.

Political barriers

In both the California Current and Gulf of Maine bioregions, several respondents indicated that discussing climate change-related ecosystem services with the people who accessed and used the coastal lands for recreation could be controversial or perceived as being off topic. In some instances, carbon services were singled out to be avoided because of the perceived political and controversial nature of climate change. An education and outreach manager in a conservation organization noted:

We don't talk about that, really... The people we get in here, they want a pretty space to walk around and explore and anything climate change—it's just way too ... it wouldn't work.

As one environmental stewardship manager in New England indicated, "The political climate is probably the biggest impediment right now." Several respondents highlighted that, in coastal systems, climate change is rarely discussed in terms of carbon mitigation, water quality impacts, or temperature change. One environmental manager noted,

Climate change comes into our work in one area only: sea level rise. We assume a three-foot sea level rise over the next 100 years. That's it.

Motivation/Identity barriers

In addition to the three barrier types listed above, several interviewees highlighted other reasons that their organizations would not engage with ecosystem services related to carbon, even through simple actions such as highlighting the benefits of restoration for carbon sequestration in their promotional materials or interpretation activities. The data suggest issues around notions of carbon and ecosystem services, which are essentially understood to be novel and perhaps not easily comprehended. As one program manager described,

Ecosystem services is not an approachable concept for most folks. It is more of a policy or management idea—not an idea that resonates with the public.

Another education and outreach coordinator added,

People don't understand ecosystem services or that you can sell the air or something. What they understand is what they see in front of them, the physical things they can touch and feel.

The data also suggest that some people engaged in conservation activities within the organization did not want to embrace these concepts, learn about them, or discuss them. This is not a barrier of expertise, but rather a distinguishable type of barrier: resistance to ecosystem services-related concepts based on the motivation and identity of conservationists. One described, for example,

There's a lot of people here who still think in terms of species—fish—rather than in terms of ecosystems. It's their habit, what they're used to, so why should they change?

Several organizations repeated this refrain: ecosystem services were opaque to the majority of those working in the practice of coastal stewardship and conservation. These individuals had motivations grounded in identities as ‘naturalists’ or ‘birders’; these motivations drew, in part, from an ecological understanding based in habitat delineation and species identification. Although these individuals purport to understand that climate change is a threat in these specific places—primarily to the life cycles of specific species—the role of ecosystem carbon sequestration in mitigating climate change does not align with the naturalist identity.

Age played an important role in the identification of this barrier. In each case, the respondents describing this barrier mentioned that those involved in land stewardship and conservation outreach were retired, in the over 55 age group, or otherwise understood to be of an older demographic:

We’ve got a lot of retired people, so part of what happens is you get people entrenched ... they’ve learned [to be naturalists], and they’re gonna teach it and you don’t get the new information finding its way in ... That’s part of what limits the scope of what we do.

Organizational staff and volunteers in this category were described as having motivations centered on the maintenance of habitat and the conservation of specific species, such as unique or endemic plants, fish, migratory birds, or commercially important species. One respondent said, for example,

For us, ‘ecosystem services’ would be about the values we get from the environment and that’s about sustaining a commercially viable fishery and not having any fish consumption advisories.

Another interviewee indicated,

I think that, for ecosystem services, we don't use that term, but we talk about values. I think that for clam flats, the value is clear. People stay working and employed, and that's economic benefits and jobs in the community. It's very clear to make the link that poor water quality means less money.

Interestingly, among organizations that were actively working on carbon-cycle ecosystem service management within their conservation missions, they expressed a broader frustration with single-species management paradigms, viewing them as impediments. As one Executive Director explained,

If you talk about a sand dune as piping plover habitat, then many people say, "I don't know what plover is, so I don't care," but if you talk about the millions of dollars of property damage averted from floods and sea level rise because of the value of the sand dunes, then people pay attention. They can relate to that.

Localism barriers

The barrier of motivation and identity is closely related to a barrier of localism. Despite the opportunities for co-benefits, several organizations highlighted a hesitation to engage in work on ecosystem carbon sequestration because they did not perceive it to be a local concern. Specifically, these organizations regarded engaging in carbon accounting of land and carbon markets as something that "big NGOs like TNC and EDF" do and not something that place-based conservation organizations with just a few employees would contemplate. The executive director of a marsh conservation organization explained that blue carbon is something that his organization is starting to have to do, not necessarily because they want to do it for their mission. He said:

You know, carbon storage in coastal wetlands only comes up in our California Environmental Quality Act (CEQA) and Coastal Conservancy reports that we

have to do. We don't really talk about it outside of that; it's not really a great way to connect with people locally.

Several respondents indicated that the entire 'carbon idea' was operating at a different scale than their conservation work, and this was why they had not considered participating or engaging. One interviewee, a science director representing a coastal watershed organization, said:

I see carbon [management] as being out of my league. As a little guy working on the ground, thinking about the carbon stored in these trees—as opposed to their caché, their cooling effect—it's just a decision being made at a different scale.

For some conservationists, the history of the specific place to be conserved and its conservation struggles was an important aspect of the mission, one apparently not fulfilled by blue carbon or similar ecosystem services. As another Executive Director said,

We talk about alewives and sturgeon and the histories of these fish, so we connect people with a history of this place as well. Carbon and nitrogen cycles don't really come into play.

While the majority of respondents highlighted concerns about place-based motivations restricting work on ecosystem services, one respondent argued that the local scale of his organization's actions made it frustrating because larger-scale governance actions and institutions were needed to facilitate work on ecosystem services. The individual described the situation in this way:

There is a really fundamental problem with the political economic system, the capitalist system, in which we live, and I think the lack of valuation of ecosystem services is a major part of it. I don't know how to change that from where I am.

Conditions under which action is taken

We have highlighted five barriers to coastal conservation organizations embracing work on carbon sequestration ecosystem services in their stewardship and conservation activities. Despite these barriers, however, some organizations reported actively engaging in work on blue carbon ecosystem services. Based on the organizations that were categorized as taking some form of 'action' on blue carbon, we developed a typology of conditions under which actions on blue carbon are taken. The two conditions necessary for action, which we found were met 100 percent of the time that actions were taken, were: (1) some form of established connection with a research laboratory or university, and (2) an expressed awareness of, or direct connection with, RAE. We consider each of these conditions in greater detail below.

Connection with research laboratory and/or university

Every organization that pursued some form of action on carbon cycle ecosystem services—whether small or more substantial—had a personal, network, or established professional connection with a research laboratory, defined as a laboratory facility with the capacity to engage in biogeochemical scientific research. While frequently the laboratory was part of a university or research institution, these connections also included non-university-affiliated research labs. Connections were often of a practical nature. For example, organizations reported actively collecting

water quality samples for analysis in the laboratory, establishing grant-based collaborations with university researchers, or employing part-time students who continued to work with laboratory researchers. As one respondent explained, “Collaboration and connections with research scientists were absolutely key.” Our data provided no indication that the connection with the research laboratory directly facilitated knowledge of ecosystem services, in general, or carbon sequestration, specifically. Rather, the connections were viewed as an avenue to providing technical expertise.

Awareness of, or direct collaboration with, Restore America’s Estuaries

Organizations taking action related to blue carbon reported one common connection: they were related in some way to the nonprofit organization Restore America’s Estuaries. In every case, conservation organizations taking action on blue carbon had immediate recognition of RAE and its work, and were generally aware of the group’s activities. As one respondent said,

So I went to a panel that was being held by the Restore America’s Estuaries group, and I got hooked in there and heard more about blue carbon ...

In a few instances, organizations reported collaborating directly with RAE on a project and seeking out RAE for their expertise with markets in particular. One respondent described,

RAE has a history of working on blue carbon, and they were the way we got involved with the broader initiatives ... The carbon market ‘hook’ went through RAE. We sought out the involvement of RAE; they didn’t seek us out. RAE had the methodology [for carbon markets].

DISCUSSION

Our results illustrate the status quo of how carbon sequestration ecosystem services resources are incorporated into the work of certain U.S. coastal conservation organizations. As ecosystem services emerge as a tool in environmental management, surfacing the barriers to incorporating ecosystem services into the conservation work of local-scale stakeholders is important in understanding the future of environmental management.

Barriers to incorporating ecosystem services

Our results illustrate that frequently identified barriers to conservation practices, such as insufficient financial or temporal resources (Eckstrom and Moser 2012) and lack of technical expertise (Hamin et al. 2014), are also barriers to conservation organizations employing the ecosystem services structure in their work.

Because carbon sequestration provides a service of climate change mitigation, incorporating these ecosystem services into the work of conservation organizations is also complicated by the politics of climate change in the United States. This phenomenon, also observed in numerous local-scale regulatory and social contexts (Clar et al. 2013), appears to be present within the context of coastal stewardship among conservation organizations, even in regions of the United States where climate mitigation policies have been enacted. Thus, the politics around climate change distinguishes carbon cycle ecosystem services from other ecosystem services. One education and outreach manager for a watershed conservation non-profit organization put it succinctly: “I wouldn’t touch carbon with a 10-foot pole.”

Bringing it back to the local scale

Scale misalignment between governance, provisioning, and benefit delivery from ecosystem services is a significant challenge in environmental management, and our data are consistent with previous work highlighting the challenges of managing ecosystem services across scales (Cash and Moser 2000; Redford and Adams 2009). Notably, our data include an instance of an attempt to overcome the barrier of scale misalignment through a re-articulation of the global-scale blue carbon ecosystem service into the local-scale domain of water quality services related to nutrient pollution. This process of re-articulation is diagrammed in Figure 3-4. As one organizational representative explained, after completing a greenhouse gas (GHG) budget for the marsh:

Blue carbon was new. It wasn't like water quality where you can feed back into the [management] community ... who are used to working on it. What do local communities know? They know eutrophication, [so there was] kind of a flip-flop of greenhouse gas work back to water quality.

Following Waring et al. (2015), this re-articulation may represent a recasting of organizational scale in an attempt to transmute the nature of the dilemma itself being worked on to one that occupies a narrower scale. In other words, regional-to-local-scale managers can manage water-quality problems relatively effectively, but they cannot manage climate change, so they may attempt to redefine the problem in local terms.

Place, not just scale, matters

One of the more surprising emergent results from our work was the degree to which the oft-cited challenge of scale misalignment is manifested through strong articulation of attachment to attributes of place. Place attachment is associated with not only land conservation, but also the preservation of a broader social-ecological landscape (Walker and Ryan 2008), as well as the preservation of values and benefits the landscape confers to a homogenous community (Chapin and Knapp 2015). Having a connection to place can positively influence landscape conservation actions, as well as other pro-environmental actions (Vaske and Kobrin 2001). Notably, the actions that individuals undertake tend to match the scale of the sense of connection to place that an individual exhibits (Ardoin 2014).

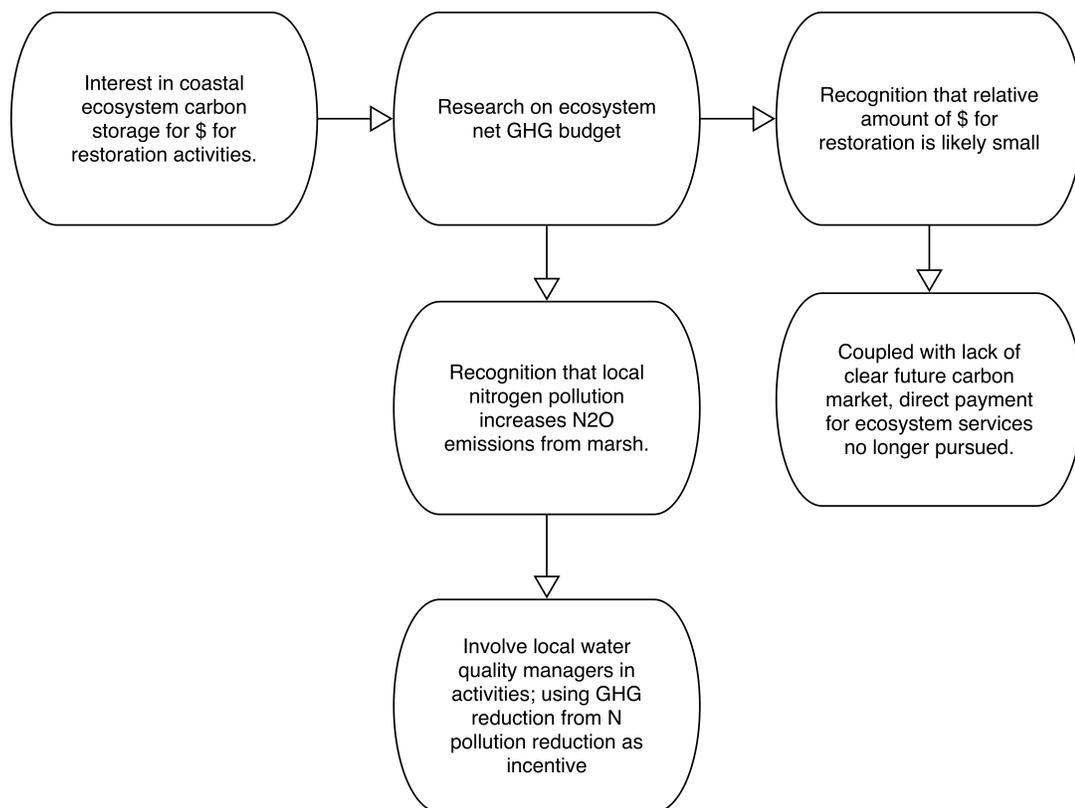


Figure 3-4. Conceptual diagram of re-articulation of greenhouse gas ecosystem services in terms of local water quality issues.

The connections that define a sense of place, regardless of scale, for an individual can be considered as having four dimensions: biophysical, psychological, sociocultural, and political-economic (Ardoin et al. 2012). In our semi-structured interviews, conservationists described connections to the places they were working to conserve across all four dimensions. Our data suggest that conservationists working in place-based, local coastal conservation organizations appear to value these connections to place over climate mitigation ecosystem services, despite the strong potential for receiving multiple co-benefits from multiple ecosystem services generated from conservation and restoration actions. The managers and conservation professionals in our sample did not regard carbon cycle ecosystem services as adequately supporting any of the dimensions of place; their responses suggested that their valuation and operationalization are seen as occurring on larger social, political, and governance scales, which do not carry with them the attributes of place that motivate conservation (Stedman 2002).

Our interviews also reveal that this place-related issue is not one that applies to individual ecosystem services conceptually, but rather one that applies to the framework of ecosystem services and, specifically, to carbon sequestration services. Ecosystem services that are delivered to local communities by place-conserving actions that are taken in those communities are, indeed, a focus of the missions of local, place-based conservation organizations. The presence of healthy populations of key resource species, recreational opportunities, affective feelings in place, and local natural history were all highlighted by interviewees.

Yet, numerous conservation professionals in our sample reported that they regarded ecosystem services as an inadequate and ineffective framework with which to approach advocacy to the general public, especially within a community of stakeholders in the resources supplied by coastal ecosystems. Chapin and Knapp (2015) have highlighted that a sense of place can be understood as an organizing concept for negotiating contested spaces of sustainability; we see evidence of that among coastal conservation organizations. Resistance to ecosystem services is not a resistance to sustainability practices, but to a certain conflicting vision of sustainability. While Chapin and Knapp (2015) highlight opportunities for local-to-global sustainability actions through expanding and multiple senses of place, we do not yet see this kind of thinking being salient for the particular communities in which the organizations in our sample work.

No interviewees said they spoke specifically about ecosystem services when communicating with the public or their supporters, and all said they found it to be best to avoid the phrase. When prompted, interviewees indicated they believed the value of a coastal ecosystem to a community would be more appropriately and compellingly framed in terms of the resource values familiar to people. Interviewees also indicated that a compelling frame might center on those resource values that are easily quantified (e.g., coastal property values, commercial shellfisheries), or perhaps in terms of aspects to which residents could easily relate, such as health. As one director highlighted,

Linking measurements of contaminants in the water to measurements in oysters is the best way to move forward on ecosystem services. The health concept linking the medical community and human health impacts to

ecosystem impacts would really let the ‘ecosystem services’ framework take off and grow.

Facilitators: expanding beyond the top-down frame

Our data strongly suggest that RAE functions as a node in the network of conservation NGOs working on coastal ecosystem services and, specifically, on blue carbon. For our sample population, awareness of RAE’s activities appears to be a necessary condition for action on blue carbon by conservation groups in the United States. These results highlight that being connected to the information-rich, networked structure of national and international NGOs and academic scientists is critical to working on blue carbon.

Taken as a whole, our results both confirm and expand upon the observation that the design and operationalization of the ecosystem services framework is top-down. Numerous workers and scholars have placed increased emphasis on polycentric, stakeholder-driven, and adaptive approaches to ecosystem management at local scales (Berkes 2009; Berkes 2010; Schultz et al. 2015). If the ecosystem services framework is to take a keystone place in the future of ecosystem management, careful attention to stakeholder agency and knowledge production, in the context of a top-down framework of dissemination, is merited.

Although our work was limited in scope to the case of blue carbon and we cannot assess whether similar conclusions would hold for the management of carbon sequestration services in terrestrial systems or for other ecosystem services, our work makes clear that local, place-based coastal conservation organizations in the United States have not yet mainstreamed the concept of ecosystem services. As the ecosystem

services concept enters a broad swathe of federal environmental decision-making, careful attention to its reception, dissemination, and evolution at local scales is essential if the institutional development being undertaken at a massive scale is to be sustained. Focusing not only on the examples of the successful incorporation of the ecosystem services frame into decision-making (Arkema et al. 2015; Ruckelshaus et al. 2015), but also specifically addressing and overcoming the barriers to the incorporation of ecosystem services into local-scale conservation practice, should be a central focus of future scholarly and practitioner attention.

Chapter 4

Carbon of convenience: The consideration of blue carbon under the National Environmental Policy Act⁴

ABSTRACT

In an effort to mainstream climate mitigation policies, there have been recent calls for the incorporation of carbon sequestration ecosystem services into existing environmental management frameworks. Because it is comprehensive in its application, the National Environmental Policy Act (NEPA) process has been highlighted as a statutory framework in which ecosystem services, such as coastal carbon sequestration, can be incorporated without the need for additional statutory development. Currently, the Council on Environmental Quality has produced draft guidance on how agencies should assess environmental impacts on carbon storage in ecosystems exists, and thus the current treatment of these impacts is takes place in a “flexible policy space”, absent finalized guidance. Because of path-dependent, first-mover effects in regulatory construction, understanding the *status quo* practice of the uptake, acknowledgement and conceptualization of carbon cycle ecosystem services in contemporary agency NEPA implementation can guide the development of future guidance and also inform our understanding of the modalities of effective adaptive management in the context of global change. Our assessment of the current inclusion, acknowledgement and quantification practice of blue carbon ecosystem services and ocean acidification impacts within environmental impact statements in the coastal

⁴ This chapter was written with the help of Anna Wietelmann, Margaret Caldwell and Michael Wara. It is being prepared for submission for publication.

zone reveals that carbon sequestration is only considered in assessments of environmental impacts when it is being restored, and not when this service is being lost. Even within single environmental impact statements, the treatment of ecosystem carbon storage is as a form of implicit offset to mitigate other construction emissions, reported when it is convenient. We also find that the use and recognition of the ecosystem services framework is rapidly increasing in coastal environmental impact statements. These results demonstrate the consequences of treating greenhouse gases as air pollutants under the Clean Air Act, and suggest need for further refined directed attention by decision-makers to the treatment of greenhouse gas sources and sinks within ecosystems.

INTRODUCTION

Adaptive Management in the Anthropocene

Anthropogenic global change, including climate change, nutrient pollution, ocean acidification and biodiversity loss, is altering earth system functioning, with potentially devastating consequences for organisms, ecosystems, communities and societies (Steffen et al. 2007). In light of this rapid anthropogenic global change, understanding how environmental governance institutions – and the decision-makers within them – use, incorporate, and shape rapidly accumulating scientific information about emergent environmental problems is a key question facing both scholars of environmental governance and sustainability scientists interested in working toward solutions to these problems (Palsson et al. 2013). Globally, the pace of production of new scientific knowledge about human impacts on the environment has far outstripped

the pace of production of new environmental laws to address these emergent problems. In the United States, innovations in environmental policy seeking to address the problems of anthropogenic global change have largely come in the form of changes in implementing regulations (Rabe 2009).

The rapid pace of environmental destruction and large uncertainties about environmental impacts of development over the last half-century led to the development of interest in more adaptive environmental management approaches, that can more flexibly incorporate new information, through experimentation, without having to redesign institutions. A substantial amount of scholarly attention has focused on the modalities of adaptive management approaches that allow for regulatory agency experimentation in environmental management programs and for the flexible incorporation of new scientific information into decision-making processes (McClain and Lee 1996; Gunderson 2001). Such scholarly attention has frequently focused on the adaptive management of natural resource use at local scales with particular attention to co-management regimes (Armitage et al. 2008) and the incorporation of traditional ecological knowledge into resource management frameworks (Berkes et al. 2000). Such research has shown that the use of adaptive management approaches can improve the environmental and social outcomes of some forms of environmental decision-making facing rapid global change under some circumstances (Tompkins and Adger 2004; McFadden et al. 2011), although others have criticized reports of successful implementation of adaptive management for being more focused on process-as-outcome than on substantive environmental improvements (Rist et al. 2013). The adaptive management approach has been formally adopted by some federal

agencies in the United States, including the Department of Interior (Williams et al. 2007).

Knowledge to Action

One of the central concerns related to adaptive management and its efficacy lies in understanding the pathways and relationships that connect scientific knowledge production to management decision-making. In particular, recent attention to the conditions under which adaptive management is successful has highlighted that the “knowledge to action” pathway is strongly mediated by the institutional networks and narrative framing of environmental problems (McGreavey et al. 2013). The characteristics of the receiving institutional governance context strongly shape how new information is used within the “knowledge system”, and what forms of new information are most effective at driving actions (Cash et al. 2003).

The question of how the institutional context of decision-making influences the successful uptake of new scientific knowledge is particularly salient for the emergent scientific problems of anthropogenic global change. Specifically, climate change and ocean acidification have rapidly emerged as salient threats to ecosystem functioning, economic livelihoods, and human well-being (Steffen et al. 2015; Eckstrom et al. 2015). Both of these problems are caused principally by anthropogenic perturbation of carbon cycling through greenhouse gas (GHG) emissions from fossil fuel combustion and land-use change. As the scientific evidence documenting anthropogenic global change has grown, agencies and decision-makers are now faced with entirely new environmental problems that were not understood, known or articulated at the time

when most environmental statutes were written. The ability of institutions to successfully respond to these problems represents a core challenge of adaptive management.

At the federal level in the United States, comprehensive legislative responses to climate change and ocean acidification have been lacking. In particular, after the failure to pass comprehensive climate change and energy legislation by the United States Congress in 2009, the Obama Administration and its federal agencies elected to use existing statutory authorities to implement regulations to address climate change and ocean acidification. The most salient example of such tailoring of existing statutes is the Environmental Protection Agency's regulation of GHG emissions as air pollutants under the authority of the Clean Air Act. This has meant that anthropogenic GHG emissions from the combustion of fossil fuels are considered to be a form of air pollution.

Accounting for Impacts and Ecosystem Services

As emergent environmental problems mature, policy responses shift from a focus on problem definition to increased attention to the documentation of the variability and intensity of the problem, including an increased focus on accounting for impacts. In climate change policy development, Rabe (2009) argues that we have now moved from the first-generation of policies focused on problem definition to a second-generation of climate change policy responses (Rabe 2009), focused on adaptation planning and the specific details of implementing of mitigation policies.

Understanding the formation of this second-generation of policy innovations in greenhouse gas accounting is critical because there are strong “first-mover” path dependent effects (Upham et al. 2014). Diffusion and uptake of regulatory approaches is strongly structured by networks (Busch et al. 2005), and once an approach is initiated institutional inertia makes it very challenging to change course. Thus, the current era of environmental policy development represents a critical juncture in shaping the future of climate governance.

Along with regulatory development for GHG emissions, the last several decades have also witnessed rising scientific recognition and economic valuation of the market- and non-market values produced by ecosystems, generally termed “ecosystem services.” (Costanza et al. 1997, Daily and Matson, 2008). The ecosystem services framework has recently begun to migrate from the realm of scholarly knowledge production to environmental governance in the context of sustainability (Maler et al. 2008). Separately from the regulatory processes under the Clean Air Act, the White House in October 2015 released a memo directing all federal agencies to include the valuation of ecosystem services within their decision-making processes (Donovan et al. 2015), and agencies are currently working to determine best practices for such incorporation (Olander et al. 2015). And of course, the use of ecosystem services in policy has implications for the production of knowledge about ecosystem services (Posner et al. 2016).

Several studies have recently elucidated pathways through which ecosystem services, and in particular those services related to carbon cycling, can be incorporated into a wide-range of other existing statutes (Ruhl 2010; Sutton-Grier et al. 2014;

Schaefer et al. 2015). In particular, Granek et al. (2010) has suggested that the ecosystem services framework could help integrate coastal-ecosystem management and Sutton-Grier et al. (2014) highlighted that ecosystem services related to coastal carbon cycling could be directly “read into” existing federal statutory authorities, including the Coastal Zone Management Act and National Environmental Policy Act.

Thus, taken as a whole, we are currently experiencing a period of rapid institutional tailoring of carbon cycle accounting paradigms in climate change policy and around the management of ecosystem services within the US Federal government, as agencies incorporate emergent knowledge of both rapid anthropogenic global change and new frameworks for assessing the value of ecosystems to human societies. The decisions, structures, and norms currently being developed are likely to strongly shape the future forms of federal climate change governance.

Here, we focus our attention on the a single case study of the consideration of the treatment of carbon cycle accounting and ecosystem services in the coastal zone: the National Environmental Policy Act (NEPA) processes. NEPA provides the governance context in which agencies must assess the total set of environmental impacts of proposed actions, and the NEPA process, and in particular the detailed and lengthy environmental impact statements produced under this process, provide an excellent case study in which to examine the treatment, understanding, and contextualization of emergent challenges associated with anthropogenic global change at this critical juncture in the knowledge-to-action pathway. In analyzing the uptake of new scientific information in existing regulatory contexts, we hope to contribute to the growing literature on regulatory diffusion of climate change policies and to help

shed light on the pathways through which scientific information about emergent environmental problems engages with management actions. We are particularly concerned with the barriers to and drivers of the incorporation of new frameworks for understanding anthropogenic global change.

The National Environmental Policy Act Process

The US National Environmental Policy Act (NEPA) is arguably one of the most significant, durable and impactful federal environmental statutes in the United States (Stein, 2010). Passed by the US Congress in 1969 and signed into law by President Richard Nixon in 1970, NEPA established a procedural requirement that all proposed federal agency actions – those activities that require permits from the federal government agencies – be accompanied by an assessment of the environmental impacts of that action. Under the NEPA process, for a proposed federal action, the lead federal agency prepares an initial Environmental Assessment. Through this initial assessment, the agency determines whether or not the proposed action being considered is likely to have any significant environmental impacts. If no significant environmental impacts are anticipated, the agency releases a Finding of No Significant Impact (FONSI), and the NEPA process concludes with the initial Environmental Assessment. If any significant environmental impacts are anticipated, the lead agency must then prepare an environmental impact statement (EIS), including a description of the impacts of the proposed action, any mitigation measures of any significant environmental impacts, as well as descriptions of the environmental impacts of alternative actions, including a no action alternative.

While an agency is not required to choose the least-environmentally impactful alternative considered in an environmental impact statement, the Supreme Court held in *Calvert Cliffs Coordinated Committee v. US Atomic Energy Commission* that the procedural requirement of preparing an environmental impact statement requires agencies to meaningfully consider the results of the EIS analysis. Nonetheless, NEPA has frequently been criticized for being an exercise in bureaucratic paper-pushing, without any substantive environmental benefits (Karkkainen 2002).

Proponents of the importance of NEPA, however, argue that such delays can be environmentally meaningful because the public ought to know about the environmental effects of government actions, and NEPA provides the process through which the public are informed (Sunstein 2015). They contend that the procedural requirement of scoping, drafting, and finalizing an environmental impact statement, with each step, under the Administrative Procedures Act, open to public comment and input, provides an opportunity to slow down decision-making and give the public time and opportunity to assess the environmental consequences of actions, and that, in so doing, public input is afforded a window in which to meaningfully participate (Glucker et al. 2013). Functionally, judicial interpretation of NEPA has also provided a pathway to successful environmental litigation, providing a legal hook to organizations and entities to litigate decision-making processes on procedural grounds in attempts to block actions (Anderson 2013).

CEQ Guidance on Climate Change

Within the Executive Branch, NEPA is implemented by the Council on Environmental Quality (CEQ) in the White House (which was created by NEPA), which promulgates regulations guiding the implementation of NEPA. During the Obama Administration, CEQ has provided two rounds of draft guidance on how agencies should consider climate change and greenhouse gas emissions within the NEPA process. The first Draft Guidance on Consideration of the Effects of Climate Change and Greenhouse Gas Emissions was released on February 18, 2010. A revised Draft Guidance was subsequently released on December 18, 2014. Formally, this guidance is still in “draft” form, meaning that the guidelines for consideration of thresholds of significance for GHG are not required regulations. The draft nature of this current guidance creates what can be understood as a “flexible policy space”, where agencies, knowing that attention to greenhouse gas emissions has developed and is developing, can choose to ignore the guidance, to follow its suggestions on how to consider GHGs, or to go above and beyond recommended methodologies. Below, we briefly review the specific content of the two rounds of draft guidance.

The 2010 Draft Guidance on the Consideration of Climate Change and Greenhouse Gas Emissions specified that federal agencies should consider both the GHG emissions associated with a proposed action, as well as the effects of climate change in relationship to the proposed action’s impacts. In particular, the Draft Guidance adopted the metric of 25,000 metric tons CO₂ yr⁻¹ from the 2009 EPA Greenhouse Gas Mandatory Reporting Rule as a metric, which should guide agencies in determining whether to quantify the GHG emissions of a proposed action. While the CEQ guidance was explicit that this was not a “threshold of significance,” but

“rather as an indicator of a minimum level of GHG emissions that may warrant some description,” it was the first time a quantitative level of emissions was listed in CEQ guidance. The CEQ Guidance also notably suggested that agencies assessing proposed actions that were likely to produce emissions at a level less than 25,000 metric tons CO₂e yr⁻¹, but over a long time duration, agencies should consider a detailed GHG analysis. The 2010 Draft Guidance excluded the need to consider the management of federal lands and emissions which might take place from land uses (CEQ, 2010).

The 2014 Revised Draft Guidance built on the 2010 Draft Guidance in several ways. First, it reiterated clear that EISs should consider both (a) the GHG emissions from an action as a cause of climate change and (b) the implications of climate change for the environmental effects of the proposed action. The 2014 Revised Draft Guidance also continued the use of the 25,000 metric tons “reference point,” again highlighting that it was not a substitute for an assessment of significance, which is subject to agency-specific consideration. Notably for our consideration of ecosystem carbon sequestration, the Revised Draft Guidance also removed the blanket exclusion of GHG emissions from lands, specifically highlighting that GHG emissions from the “destruction of natural GHG sinks such as coastal wetlands and forests, and the loss of future sequestration” should be considered and analyzed as GHG emissions (CEQ 2014).

Blue Carbon, Ecosystem Services and Ocean Acidification

In this study, we consider the case of the treatment of three emergent areas of global change scientific knowledge within NEPA: the treatment of (1) blue carbon –

the sequestration of carbon within coastal ecosystems, (2) other coastal ecosystem services – the market and non-market values produced by ecological functions within ecosystems, and (3) ocean and coastal acidification – the decreasing pH and changing carbonate chemistry that threatens marine organisms, including culturally and commercially important calcifying organisms. We focus in particular on the management of coastal rather than forest carbon cycling because the methodologies for its accounting have been less fully developed than those for forests, and because the agencies involved in coastal zone management present more varied institutional contexts in which to study the variation in the incorporation of new scientific information than the US Forest Service.

Blue Carbon

Blue carbon refers to the sequestration and storage of atmospheric carbon in tidal flats, sea grass meadows, coastal mangrove forests, salt marshes and tidal wetlands, and kelp forests (McLeod et al. 2011). Such ecosystems globally sequester 100s of millions of tons of CO₂ annually, making them among the most powerful carbon sinks per unit area on the planet (Chmura et al. 2003) Coastal ecosystems are particularly powerful carbon sinks because of low rates of organic material decomposition and oxidation due to inundation and because of the high capacity of blue carbon ecosystems to trap allochthonous organic carbon (Greiner et al. 2013). Globally, threats to blue carbon ecosystems include habitat destruction for alternative coastal land uses such as shrimp farming and urban development, sea level rise and

storms associated with climate change, and dredging activities which remove sea grasses and other ecosystems (Pendleton et al. 2015).

Other Coastal Ecosystem Services

Including the values of ecosystem services into decision-making has frequently been suggested as a pathway through which the non-monetized benefits of well-functioning ecosystems can be valued in cost-benefit analyses (Daily and Matson 2008), though it has been criticized by conservation biologists for being an exercise in optimization that may lead to negative environmental impacts (Redford and Adams 2009). Classically, ecosystem services – the market and non-market values to human societies that ecosystems generate – are separated into four categories: supporting services (e.g. habitat protection), regulating services (e.g. carbon sequestration, water quality maintenance), provisioning surfaces (e.g. food production), and cultural ecosystem services (e.g. spiritual sense of place and recreation). Quantification of the value of ecosystem services has emerged as a rapidly-developing scientific field, though some ecosystem service categories have resisted simple value quantification (Olander et al. 2015).

Ocean and Coastal Acidification

Globally, anthropogenic CO₂ emissions are increasing atmospheric CO₂ levels, which increases the amount of CO₂ entering the ocean, driving changes in ocean carbonate chemistry and lowering global surface ocean pH (Orr et al. 2005; Doney et al. 2009). These global changes in ocean chemistry can have negative impacts on

ecologically, culturally, and commercially important shell-forming organisms, including corals, such as clams and lobsters (Kroeker et al. 2013). OA also threatens fisheries through disruptions of the food-web (Branch et al. 2013) and by affecting the sensory environment of the ocean (Rossi et al. 2016). Nutrient pollution from suburban stormwater and agricultural runoff can also raise coastal CO₂ concentrations by stimulating phytoplankton production and remineralization, exacerbating acidification in coastal waters (Strong et al. 2014, Gledhill et al. 2015). Colder coastal waters that receive high inputs of low-alkalinity freshwater are particularly chemically vulnerable to the impacts of acidification (Salisbury et al. 2008, Mathis et al. 2015). Such coastally-enhanced acidification creates hot-spots of low pH environments in specific coastal areas (Strong et al. 2014). Through the impacts of ocean and coastal acidification (OCA) on socially and economically important resources, OCA threatens the livelihoods of many coastal communities (Eckstrom et al. 2015). Recent scientific evidence has also suggested the potential for mitigating actions to reduce coastally-enhanced acidification (Kelly and Caldwell 2013). Mitigation approaches include carbonate restoration through treatments with shells or restoration of carbonate-preserving coastal ecosystems like sea grasses (Hendricks et al. 2014) and potentially the use of localized engineering solutions (Kowweek et al. 2016).

By assessing how coastal carbon sequestration, ocean acidification and coastal ecosystem services are considered within current NEPA processes, we hope to contribute to the understanding of the modalities of adaptive ecosystem management under global change, and to help shape future institutional development and policy

guidance as the incorporation of climate change, ecosystem services, and ocean acidification into federal-decision making matures.

METHODS

To assess the status quo of the consideration of coastal ecosystem carbon sequestration, other coastal ecosystem services, and ocean acidification within the flexible policy space of contemporary NEPA processes, we analyzed coastal EIS documents produced over a three-year period from 01 October 2012 to 01 October 2015. A record of all Final, Draft, Final Supplemental and Draft Supplemental EIS documents published during this period was obtained from the US Environmental Protection Agencies' searchable database of EIS documents (<https://cdxnodengn.epa.gov/cdx-enepa-public/action/eis/search>). "Coastal EIS documents" were defined as environmental impact statements produced to assess the impacts of federal actions taking place in the "immediate coastal zones" of 24 coastal states and five coastal territories, in the state offshore waters of these states, or in the federally-administered territorial waters and Exclusive Economic Zones of these states, out to 200 nautical miles from shore. For the purposes of our analysis, states that are considered coastal states under the National Coastal Zone Management Program, but which only have coastlines along the Great Lakes (Minnesota, Michigan, Wisconsin, Illinois, Indiana, and Ohio) were excluded from analysis. For the two states with both oceanic and Great Lakes coastlines – New York and Pennsylvania – the coastal zones were defined as only those coastal zones along the Atlantic Ocean and Delaware River Estuary, respectively. Under the National Coastal Zone

Management Program, the area of the coastal zone is defined separately by each coastal state, ranging from just 100 feet inland from the shoreline, to the areas of all towns/municipalities with coastlines, to the entire state, to other definitions based on specific elevation or hydrologic features.⁵ In order to isolate environmental impact statements produced for federal actions that had a strong potential to influence carbon cycling in coastal ecosystems (and in order not to include, for example, EISs written for federal office building construction 100s of miles inland from the coast), we used an operational coastal zone definition, which we term “the immediate coastal zone” to determine which EISs were “coastal EIS documents”. The “immediate coastal zone” was considered to be the area that is both (a) within that state’s own defined coastal zone, and (b) also within 1000m of the mean high water line, or 1000m of the head of tide in tidal estuaries, or otherwise located offshore. Distances from the shoreline and head of tide were checked using Google Earth. This two-part definition meant that some activities in major metropolitan centers (New York City and San Francisco) that were within 1000m of the shore, but outside of that state’s own coastal zone definition, were not included in our analysis.

Electronic Portable Document Format (PDFs) of all EIS documents were obtained. The majority of full EIS documents, including all volumes and appendices, were downloaded in PDF form directly from the EPA database website. For documents that were listed in the database, but for which no PDF was available from the EPA website, internet searches were performed until the relevant PDF document

⁵ Alaska is the only coastal state that does not currently have a Coastal Management Program and thus does not have an officially defined coastal zone under the program. However, prior to 2011, when the state coastal program expired, Alaska had a defined coastal zone and set of coastal zone management districts, which were considered Alaska’s coastal zone here.

could be obtained. All of the draft and final EIS documents analyzed were drafted and opened for public comment after the publication of the February 2010 Draft CEQ Guidance on Climate Change GHG Emissions, while some documents were produced prior to, and some after, the release of the December 2014 CEQ Revised Draft Guidance.

The consideration of coastal ecosystem carbon cycling in EIS documents was analyzed using NVivo Qualitative Analysis Software (QSR International). All coastal EIS PDF documents were imported into NVivo. These documents were initially categorized based on the metadata included within the EPA database. These metadata categories for each EIS document were: (1) the document type (final or draft EIS), (2) the title of the document, (3) the date its release was published in the Federal Register, (4) the date that the public comment period closed, and (5) the lead federal agency in charge of the EIS.

Using a modified grounded theory approach (Corbin and Strauss 1990), we developed a typology of the kinds of federal actions that were triggering NEPA processes within the coastal zone. The typology was exclusive and fully categorical, meaning that every EIS was assigned to one and only one category. Each EIS was assigned to one of 9 categories of types of actions, which were relatively evenly distributed within the dataset. These categories were:

1. **Land Management or Conservation Management Plans** (often for specific Wildlife Refuges, National Parks, or other land areas, including sale or acquisition of land).

2. **Energy Projects** (Ocean drilling leases, natural gas distribution or pipeline projects).
3. **Construction Projects** (Primarily the construction or modification of bridges, roads, piers and port facilities.)
4. **Military Operations** (Primarily US Navy and other military training and testing exercises, or the relocation of military aircraft)
5. **Dredging Activities** (Harbor expansion or maritime transportation improvement projects.)
6. **Water or Flood Management Projects** (Flood mitigation, shoreline improvement, re-vegetation, or other water diversion projects).
7. **Fisheries Management Plans** (Management of specific harvested marine living resources)
8. **Species Management Plans** (Management of specific species of concern, often threatened or endangered species under the Endangered Species Act).
9. **Ecosystem Restoration Plans** (Similar to land conservation management plans and water or flood management projects, these projects were specifically focused on restoring ecological functions.)

All references to ecosystem carbon cycling and greenhouse gas emissions within coastal EIS documents were analyzed, categorized and contextualized individually, following a serial analysis approach. First, all references within these documents to “carbon”, “CO₂”, “greenhouse gases”, “GHGs”, “sequestration”, “sequester”, “carbon sinks”, “ecosystem services”, “ecological services”, “blue

carbon”, “climate change”, “ocean acidification”, and “carbon footprints” were searched, recorded, coded and stored for analysis.

References to any emissions of greenhouse gases were then analyzed for whether they referred to emissions from the combustion of fossil fuels, or emissions from the loss of ecosystem carbon, whether the amount of greenhouse gas emissions from the proposed action was quantified numerically or not, and whether or not reference was made to Draft CEQ guidance on the consideration of greenhouse gases. The specific section or sections of the EIS document in which both sources of GHG emissions and the impacts of climate change were discussed were also recorded.

References to carbon sequestration ecosystem services were analyzed based on what ecosystem type was involved in sequestration, and whether reference was made to activities diminishing or enhancing the sequestration potential. We also assessed whether quantified GHG totals included reductions in emissions from carbon sequestration as “offsets”, either qualitatively or quantitatively. Any references to “blue carbon” were also analyzed for what ecosystem types were considered. EIS documents were also searched to determine if any blue carbon ecosystems (sea grasses, tidal flats, salt marshes, mangroves, or kelp forests) were likely to be impacted by the proposed actions, and whether or not impacts to these ecosystems were discussed in the EIS document, regardless of whether discussion of carbon cycling was included.

Our dataset also offers an opportunity to assess the status quo of the use of ecosystem services within a federal agency decision-making context. As such, we assessed whether an EIS referred to ecosystem, environmental, or ecological services,

and whether there was any quantification or qualitative description of non-market values of ecosystem services either being lost or enhanced due to the triggering federal action.

Finally, any and all references to ocean acidification, or other changes in ocean pH or chemistry as a result of human actions, were analyzed to determine if there was more than a minimal reference to OA, what impacts of OA, if any, were documented, if there was any discussion of the phenomenon of coastally-enhanced acidification, or any possible remediation or other mitigation measures that were mentioned, and in what section or sections of the EIS OA was discussed.

In order to compare the treatment of forest ecosystem carbon sequestration with the treatment of coastal ecosystem carbon sequestration, we also randomly sampled 20% of the 236 EIS documents with the US Forest Service as lead agency during the same period, 01 October 2012 to 01 October 2015. We stratified the total population of these EISs by state and then randomly selected 46 EISs from the total population, proportionally by state. The consideration of “carbon sequestration” and “carbon sinks” within this random sample was then analyzed.

Our analyses were primarily interested in correlational trends among EIS references and phenomena, rather than causal regression analysis. Expected-value based analyses, using Pearson’s X^2 test for expected distributions, were performed to determine if statistically significant variations existed in the consideration of GHG emissions, carbon sequestration, ecosystem services and ocean acidification within coastal EISs, among lead federal agencies, federal action types, over time, of across

spatial distributions within the United States. All statistical tests and analyses were performed using the R statistical software package (www.r-project.org).

RESULTS

A total of 1,042 environmental impact statements were published in the federal register and/or had open public comment periods open during the three years between 01 October 2012 and 01 October 2015. Of these, 455 were Draft EISs, 443 were Final EISs, 93 were Draft Supplemental EISs, and 51 were Final Supplemental EISs. The distribution of all EISs between the top ten lead agencies for these EISs is shown in Figure 4-1, with the most frequent lead agencies for proposed actions being the US Forest Service (USFS), the US Army Corps of Engineers (US ACE), and the Bureau of Land Management (BLM), together comprising 44% of all EISs.

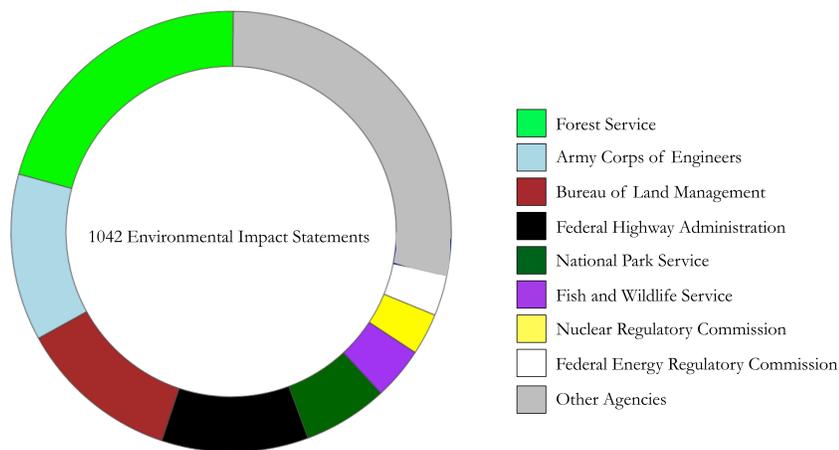


Figure 4-1. The breakdown of all EIS documents from October 2012 to October 2015 by lead federal agency.

Of the 1,042 total EIS documents during these three years, 60% (n=626) were statements for proposed actions taking place wholly or partially within the 29 oceanic coastal states and territories, or within state or federal waters. 35% (n=220) of these 626 EIS documents from coastal-states were for proposed actions taking place within the “immediate coastal zone” as defined above and were therefore considered “coastal EISs”. Thus, a total of 21% of all of the EIS documents produced during this three-year period were “coastal EISs,” for which we consider there to be strong potential for significant environmental impact to coastal ecosystem carbon cycling. (Of course, there is always potential for environmental impacts to the coastal zone from proposed actions taking place outside of the coastal zone, but our analysis here is limited to actions taking place in explicitly marine or coastal spaces.)

EIS document production during the three-year sampling window was at a rate of approximately seven EIS documents per week, and there was a slightly, but significantly negative trend for total EIS production over the three years (Figure 4-2). There is no long-term negative trend in the rate EIS production between 1987 and 2016, but, EIS production has been declining since 2006. For coastal EISs, the rate of production was approximately five to six coastal EISs per month over the three year period, a rate that was static over the three years. Of the 220 coastal EISs in our database, 21% (n=45) were published in the federal register and/or open for public comment after the release of the December 2014 CEQ Revised Guidance on the Consideration of Climate Change and Greenhouse Gas Emissions.

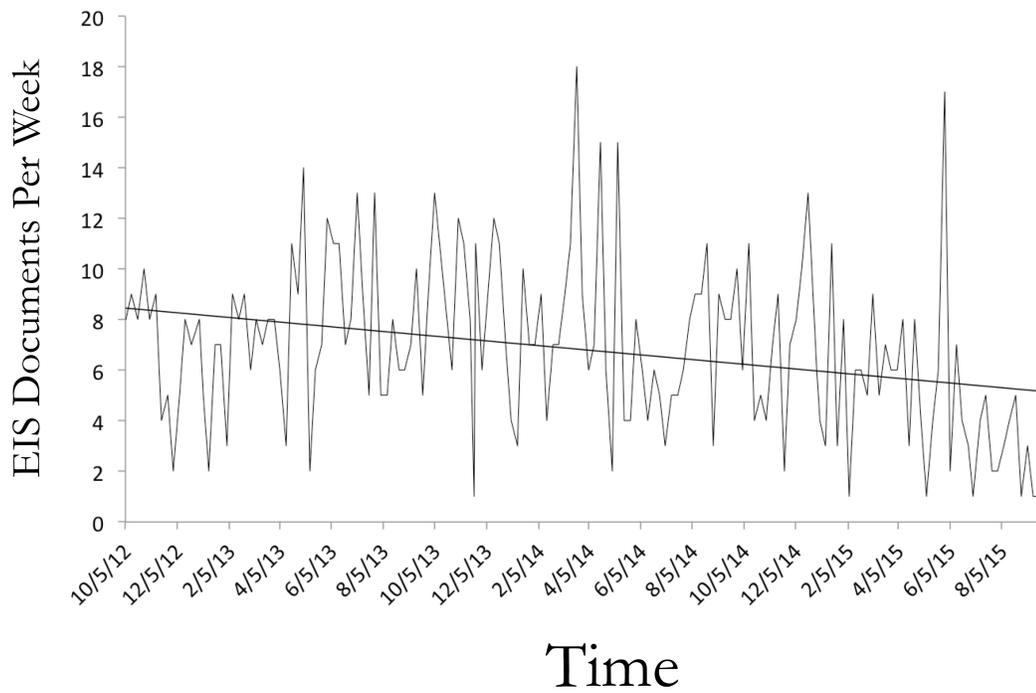


Figure 4-2. The weekly frequency of all EIS documents published in the federal register from October 2012 to October 2015. There has been a significant decline in EIS publication rate during this time. The current decline began in 2006. Overall, from 1987 to 2016, there has been no trend in the rate of EIS publication.

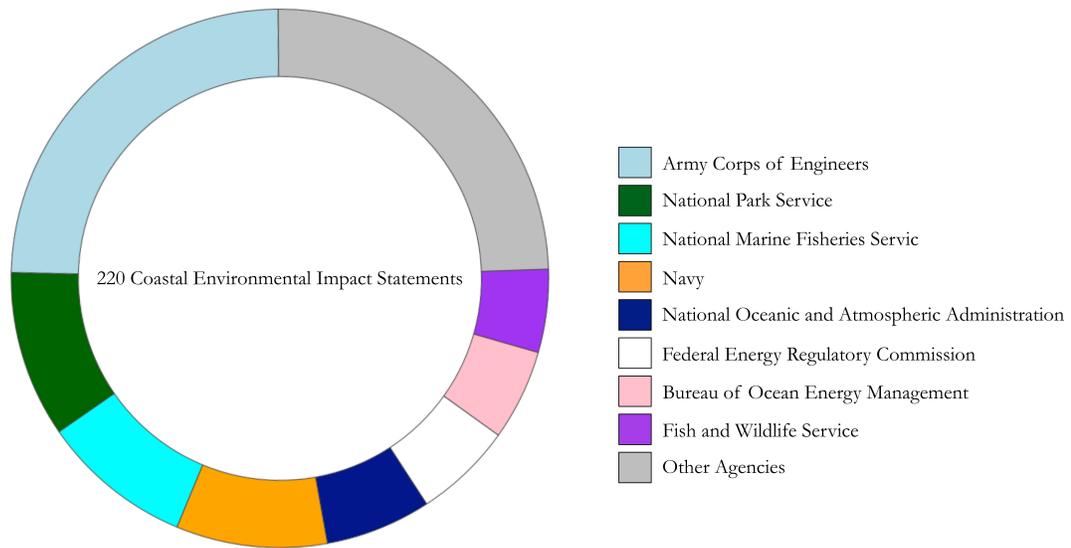


Figure 4-3. The breakdown of coastal EIS documents from October 2012 to October 2015 by lead federal agency.

The distribution of the 220 coastal EIS documents across lead federal agencies was significantly different than that of the total population of all EISs, and is shown in Figure 4-3. For example, the US Forest Service, Bureau of Land Management, and Federal Highway Administration played a less prominent role as lead agencies for coastal-zone and off-shore EISs. 70% of all coastal EIS documents were produced for proposed actions overseen by just seven federal agencies: the US Army Corps of Engineers, the National Park Service, the National Marine Fisheries Service, the US Navy, the National Oceanic and Atmospheric Administration (separately from NMFS), the Bureau of Ocean Energy Management, and the US Fish and Wildlife Service. These seven agencies fall entirely under the Department of the Interior, the Department of Commerce, and the US military.

What kind of federal actions trigger coastal EISs? Among the nine project types identified using a modified grounded-theory approach, there was a relatively even distribution of coastal EIS documents. Energy Projects and Construction Projects and Land/Conservation Management Plans were the most common types. A breakdown of all coastal EISs by project type is shown in Figure 4-4. Project types were strongly correlated with lead agency involvement. For example, Military Operations were the domain of the US Navy, US Army, US Air Force, and US Marine Corps, while Fisheries Management Plans were the domain of the National Marine Fisheries Service, and Dredging Projects were largely the domain of the US Army Corps of Engineers.

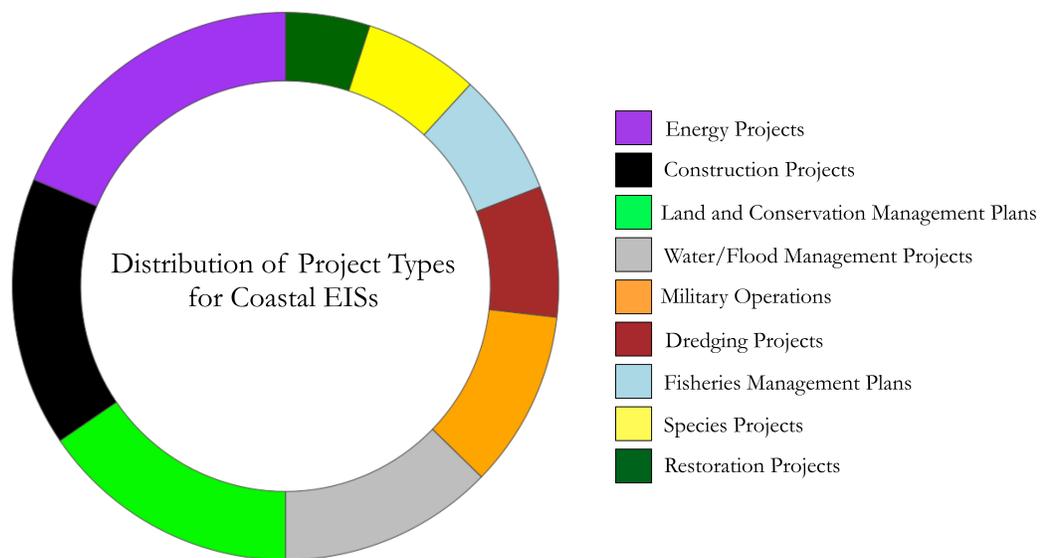


Figure 4-4. The distribution of types of actions triggering coastal EISs.

Geographic Distribution

Figure 4-5 shows the distribution of all coastal EISs around the coastal zone of the United States. EISs were not distributed evenly across the 29 coastal states and territories, nor were they proportional to population or length of coastline. Instead, coastal EIS documents were heavily concentrated on the west coast of the United States and in the Gulf of Mexico. The five states with the greatest number of coastal EIS documents were, in order, California, Florida, Alaska, Washington, and Louisiana. A total of 89 EISs were located on the Pacific (or Arctic) coastlines of Alaska, Washington state, Oregon, California, and Hawai'i, and 39 were in states with coastlines on the Gulf of Mexico. 31 EISs were in the South Atlantic Bight (Atlantic Coasts of Florida, Georgia, South Carolina and North Carolina), 18 were in the Chesapeake, Delaware Bay and Long Island Sound region of the Mid-Atlantic states, and the New England states saw just nine coastal EISs in Massachusetts, Maine and New Hampshire. Seven EISs were in the territories of Guam, the Northern Marianas and Puerto Rico. 27 coastal EISs were multi-state or programmatic EIS not based within a single state.

Coastal Environmental Impact Statements October 2012 - October 2015

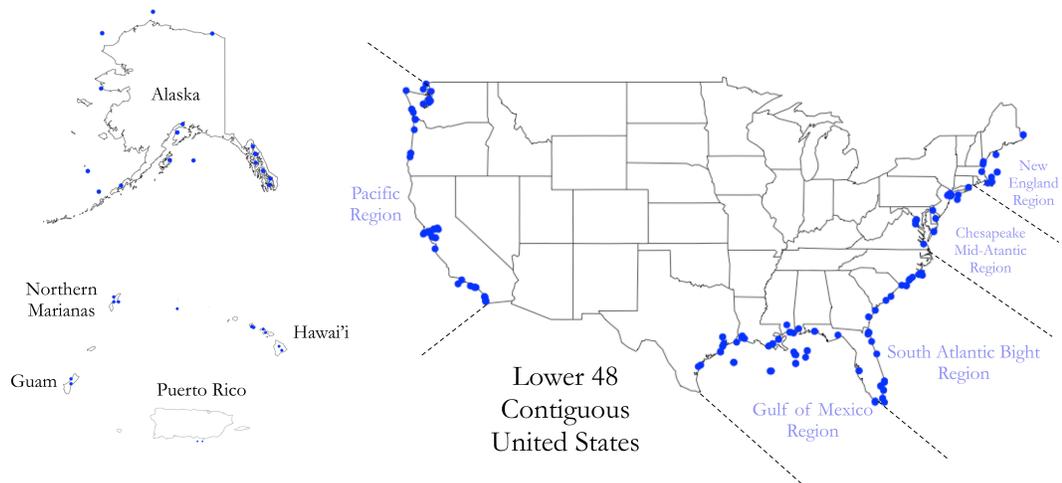


Figure 4-5. The geographic distribution of the proposed actions triggering coastal EISs. Each blue dot represents a single EIS document. Multi-state and programmatic EISs are not shown, including many fisheries management plans.

Reporting Emissions/Calculating Emissions (Responding to Draft Guidance)

Of the 220 coastal EIS documents in the dataset, 108 (49%) quantified anticipated project greenhouse gas emissions, in tons CO₂e, for different project alternatives. Of these 108 EISs with quantified GHGs, 75 EISs listed the 25,000 tons CO₂e per year reference point for GHG emissions proposed under the Draft 2010 CEQ Guidance, and numerous EISs for proposed actions in California also listed California specific significance thresholds for GHG emissions adopted under the implementation of the California Environmental Quality Act. Of these 75 EISs which listed the CEQ GHG “threshold” from the draft guidance document, only 21 EISs described anticipated project CO₂e emissions as having a significant impact, in each case

because anticipated emissions surpassed the threshold of 25,000 metric tons per year⁶. All of these 21 EISs were for military operations, energy projects, or construction/dredging projects associated with port activities. Other EISs exceeded the 25,000 tons yr⁻¹ threshold, but did not describe emissions as significant globally. Some projects, especially renewable energy projects, highlighted greenhouse gas emissions reductions under project alternatives.

A total of 87 coastal EISs quantified GHG emissions for proposed action alternatives, but did not indicate that these emissions represented significant environmental impacts either because they did not pass the 25,000 metric ton CO₂e threshold, or because they were deemed to be insignificant in comparison with total global GHG emissions. The total quantified GHG emissions for these 87 projects (selecting the highest emitting alternative for each one and comparing that with the baseline of no action) was an annualized total of 5.9 million metric tons CO₂e. Using the federal government's 2015 social cost of carbon using a 3% discount rate (<https://www3.epa.gov/climatechange/EPAactivities/economics/scc.html>), these emissions, which are those that were considered “not significant” from the fraction of coastal environmental impact statements that provided quantified emissions estimates – would be anticipated to cause \$218 million (in 2007 dollars) in economic damages from the effects of climate change.

As shown in Table 4-1, the agencies which over-performed the average rate of greenhouse gas emission quantification (~50% of all coastal EISs) were the Bureau of Ocean Energy Management (91%), the Federal Energy Regulatory Commission (85%)

⁶ There were some EISs which reported estimated quantified GHG emissions of >25,000 metric tons yr⁻¹ but which did not indicate that these should be considered “significant.”

and the US Navy (75%), the Army Corps of Engineers, US FWS and NPS quantified emissions at a rate near average, while NOAA and NMFS rarely quantified emissions. Most other agencies quantified emissions at a rate of about 50%. The agency rate of uptake of GHG quantification within coastal EISs was strongly related to the project-type under consideration. Emissions were quantified for energy projects and military operations quantified 88% and 80% of the time respectively, while Species and Fisheries Management Plans did not quantify GHG emissions. Geographically, this meant that the highest proportions of reporting of GHG emissions in coastal EISs were in states where energy and military project types were common: Alaska and Texas. The states with the highest rates of quantification of GHG emissions for non-Energy and non-Military project EIS documents were California (83% of non-energy/military) and Washington state (50% of non-energy/military).

Table 4-1. Frequency of GHG emissions quantification in coastal EIS documents by federal agency

Agency	Total # EIS	Expected # To Quantify	Actual # To Quantify
US ACE	54	27	28
NPS	22	11	7
NMFS	20	10	2
US Navy	20	10	15
NOAA	14	7	0
FERC	13	6	11
BOEM	12	6	11
US FWS	11	5	5
Other	54	27	29
TOTAL	220	108	108

Carbon Sequestration and Blue Carbon

A priori, the proposed actions being considered within all 220 coastal EISs could be anticipated to have some potential significant environmental impact on coastal ecosystem carbon cycling, even if quantification were difficult or impossible given the current state of the science. The 2010 Draft CEQ guidance provided no specific guidance for how to document such impacts, indicating that they could be removed from consideration. The 2014 Revised Draft Guidance, however, suggested that emissions from impacts on coastal ecosystems should be given consideration.

Over the three years of EIS documents in our database, a total of 27% (n=59) of coastal EISs mentioned ecosystem carbon sinks or geological, ecological, or biological carbon sequestration of any kind. 17% of all (n=37) coastal EISs included a description of environmental carbon sequestration that was specific to the situation of the EIS (while 22 included only a general discussion of global terrestrial or ocean carbon sinks in background sections on global climate change). Of these 37 EISs with site-specific discussions of carbon sequestration, 84% (n=31) included discussions of actions or opportunities to restore or enhance carbon sequestration in ecosystems. 17 of these “restoration of ecosystem carbon sink” EISs described enhancing forest carbon sinks or tree planting activities to enhance carbon sequestration, and 14 included a discussion of restoration of blue carbon ecosystems that would increase carbon storage in ecosystems.

87% (n=27) of EISs that included a discussion of restoration of carbon sinks of any kind (blue carbon or tree planting), included at least a qualitative description of how this enhanced sink would mitigate or offset greenhouse emissions from project

construction or other fossil fuel combustion. This qualitative mitigation discussion generally proceeded as follows. For example, as one coastal EIS from Oregon described:

The Hall Slough alternative would also allow for gains in additional climate mitigation benefits through carbon sequestration. For this alternative, where 90 acres of wetland would be restored, this suggests as much as 88 tons of carbon could be sequestered per year in the restored marsh area. Over the long term, carbon sequestration from the project could be expected to offset CO₂ produced during construction, providing minor, regional, beneficial effects due to carbon capture in marsh biomass.

And as an EIS from the Florida Everglades discussed:

Over the long-term, rehydration of peat soils in WCA 3A will capture many more tons of CO₂ than that emitted during construction or as a result of pump operations.

In contrast with the relatively high-frequency of the use of restored carbon in qualitatively or quantitatively offsetting other GHG emissions from projects within coastal-EIS, of the 37 coastal EISs with discussions of site-specific carbon sequestration, only 43% (n=16) described losses of carbon sequestration or GHG emissions from ecosystems. All but one of these also included discussions of restoration of sinks (i.e. they included ecosystem carbon in both terms of losses and sinks). Seven of these 16 EISs only described losses of tree or forest carbon, and, despite other descriptions of other environmental impacts related to blue carbon ecosystems, did not discuss implications of activities for carbon sequestration in these ecosystems. In total, only 10% of all coastal EISs included any discussion (whether about impacts to, restoration of, both or neither) of the carbon stored in coastal ecosystems (i.e. blue carbon), and only four EISs actually used the term “blue carbon”.

The 2014 Revised Draft Guidance, including its specific mention of carbon storage in coastal ecosystems, did not seem to influence the rate of consideration blue carbon, as just 9% of EIS documents issued after the guidance was released included reference to blue carbon.

Blue carbon ecosystems that were discussed included salt marshes, sea grass meadows, mangroves, tidal fringing wetlands, kelp forests, and mudflats. Of the nine EISs that described losses of carbon or GHG emissions from blue carbon ecosystems, six included reference to oxidation of peat soils due to a reduction in inundation or the emissions of methane from coastal wetlands. In other words, these six EISs focused on changes in the anaerobic environment that would affect GHG emissions positively or negatively in coastal wetlands. Three EISs described losses of carbon sequestration from the destruction of salt marsh habitat. In every instance where a coastal EIS included a quantification of blue carbon ecosystem carbon sequestration, that EIS was for a project that was involved in restoring or enhancing coastal ecosystem carbon sinks, rather than for a loss of carbon sequestration value. There were no EISs that only included losses of C sequestration within their accounting of CO₂e impacts. Thus, engaging in some kind of enhancement of carbon sequestration through restoration could be considered a necessary condition for the quantitative consideration of ecosystem carbon storage impacts.

The consideration of blue carbon – carbon sequestration in coastal ecosystems – in coastal EISs was strongly shaped by project type and lead agency. Of the 21 EISs that included any discussion of coastal ecosystem carbon storage, 15 were restoration, water/flood management, species, or land/conservation management projects, 3 were

military operations, 2 were energy projects and 1 was a dredging project (1 out of 17 total dredging projects discussed blue carbon). Blue carbon in EISs was dominated by the US ACE, NOAA, USFWS, NPS and the US Navy. The principal coastal energy agencies (FERC, BOEM) did not mention blue carbon. Interestingly, for the US ACE, blue carbon was considered in far more water/flood mitigation projects than in dredging projects.

Geographically, blue carbon was not mentioned in any of the dozens of South Atlantic Bight state coastal EISs, and in only one EIS from the Chesapeake/Delaware/Long Island region. Blue carbon was considered in EISs in the Pacific Coast and Gulf of Mexico at a frequency similar to the relative proportion of total EISs from those regions: while 40% of all EISs were from Pacific coast states, 43% of blue carbon EISs were from the Pacific coast, although Hawai'i and Alaska were not represented, and while, 20% of EISs were from the Gulf of Mexico region, 28% of blue carbon EISs were in the Gulf of Mexico. Most over-represented were the New England states, which, despite being the location of only 4% of all coastal EISs, had 33% of EISs consider blue carbon in their discussion of blue carbon impacts.

In contrast to blue carbon, in our sample of US Forest Service EIS documents, site-specific forest carbon sequestration and storage was considered at a significantly higher rate (43% of USFS EIS) than it was for coastal ecosystem carbon sequestration (17% of all coastal EIS).

Ecosystem Services

Of the 220 coastal EISs, 32% (n=70) mentioned the terms ecosystem or ecological services, or described other non-market values of ecosystems. There was a strong and significant positive trend in the use of the phrase ecosystem services in coastal EISs over time, even within the short time-frame of our dataset, as the phrase was used in 13% of coastal EISs in 2012, 24% of coastal EISs in 2013, 39% of coastal EISs in 2014, and 62% of coastal EISs in 2015.

The ecosystem services described in coastal EISs are numerous, ranging from carbon sequestration, to flood mitigation, to nutrient retention, to recreational and cultural ecosystem services. Discussions of ecosystem services appeared in EIS sections outlining biological impacts, species, environmental impacts to ecosystem functioning, and social and economic impacts. All of these descriptions were qualitative: within the 70 coastal EISs that included reference to ecosystem services, there were no attempts at dollar valuation of non-market ecosystem services, although numerous EISs included references to these values and highlighted that quantification would be difficult.

If we take as an expected value that roughly 1/3 of EISs include some reference to ecosystem services, we can assess whether specific project types, agencies, and geographies are under- or – over-represented in their use of ecosystem services. Restoration Projects and Land/Conservation Management Plans were highly and significantly ($p < 0.05$), over-represented in the set of coastal EISs discussing ecosystem services, with 82% of all Restoration Projects and 50% of all Land/Conservation Management Projects discussion ecosystem services. Construction and Dredging Projects were the most under-represented in the use of ecosystem

services (11% and 18% respectively), while Military, Fisheries Management, and Energy Projects were close to the overall expected average usage of the phrase. Because of this, the agencies that most frequently invoked the ecosystem services framework were the US Fish and Wildlife Service (64%), the National Park Service (50%) and NOAA (50%), while the Federal Highway Administration and Federal Transit Administration did not once use the term “ecosystem services” across 15 environmental impact statements totaling thousands of pages.

Geographically, the only anomaly in the distribution of the use of the ecosystem services framework was the Gulf of Mexico. Of the 39 Gulf of Mexico coastal EIS documents (not including multi-state offshore EISs within the Gulf of Mexico), only 2 included reference to ecosystem services, while all other geographies had similar rates of usage, slightly above the overall average.

Interestingly and anecdotally, there is still some tension and/or misunderstanding within the consultants and agency drafters of EIS about how to understand the “service” of ecosystem services, as several EISs described ecosystem services being provided to the ecosystem, rather than to human communities or societies.

Ocean and Coastal Acidification

Of the 220 coastal EISs, 25% (n=55) mentioned the impacts of ocean acidification (OA), whereas 75% (n=165) included no mention of OA. Consideration of impacts from OA was limited to a discussion of OA as a globally-occurring phenomenon that would cause impacts to biological communities relevant to the

proposed action alternatives, but unrelated to the emissions that were occurring due to the proposed action. In other words, unlike for description of climate change, OA was never considered to be an impact resulting from the proposed action, but rather a phenomenon that might have environmental impacts on the biological resources considered within the EIS.

The biological resources affected by OA ranged from corals, to fish species, to marine mammals, to sea turtles, to commercially harvested shellfish. In numerous coastal EIS documents, the discussion of OA was short and un-detailed, amounting to little more than a mention of OA or inclusion of a short reference to a scientific paper (n=19). Only two EIS documents included mention of non-global atmospheric CO₂ drivers of coastally-enhanced OA, including changes in freshwater delivery, nutrient-driven acidification, despite the possibility of actions having effects on coastally-enhanced acidification. Discussions of possible mitigation of OA through carbonate restoration or preservation was limited to two EISs, both from NOAA.

Federal agencies were not equally likely to discuss OA. We estimated an expected distribution of EISs that mentioned OA, based on the fraction of total EISs that mentioned OA (25%) and based on the distribution of total EISs across agencies (Table 4-2). Despite only representing 34% of all coastal EISs, EISs with lead agencies NMFS, NOAA, BOEM, and the US Navy significantly over-performed the expected frequency of discussion of OA and represented 68% of all coastal EISs that mentioned OA. The US FWS and NPS both included references to ocean acidification at frequencies proportional with their totals, while only 6 of 51 coastal EISs from the US ACE referred to OA impacts. Pearson's chi-square revealed that the distribution of

references to OA across agencies was significantly different than the overall distribution of coastal EISs among agencies ($X^2=38.3$, d.f. =9, $p<0.01$). Over the three years included in the dataset, there was no change in the frequency of the inclusion of ocean acidification in environmental impact statements ($p>0.10$).

Table 4-2. Frequency of ocean acidification inclusion in coastal EIS documents

Agency	Total # EIS	Expected # w/OA impacts	Actual # w/OA impacts
US ACE	54	14	6
NPS	22	5	4
NMFS	20	5	11
US Navy	20	5	8
NOAA	14	4	9
FERC	13	3	0
BOEM	12	3	9
US FWS	11	3	4
Other	54	14	4
TOTAL	220	55	55

We found a statistically significant, positive relationship between the inclusion of OA in an EIS and the inclusion of a reference to ecosystem services ($X^2=8.21$, d.f.=9, $p<0.01$). Whereas only 32% of all coastal EISs included language discussing ecosystem services, 55% of EISs that referred to OA impacts included language discussing ecosystem services. There was no positive correlation, however, between the inclusion of OA impacts and the inclusion of discussions of ecosystem carbon sequestration or blue carbon, as the number of coastal EISs that also discussed carbon sequestration (27%) was similar to the fraction of total coastal EISs referring to ecosystem carbon sequestration (31%).

Geographically, EISs that referred to ocean acidification were concentrated in the Pacific Ocean along the west coast of the United States. 65% of EISs referring to OA were from NEPA processes in Washington, Oregon, California, Alaska, Hawai'i, Guam, or the Northern Mariana Islands. Of the sixteen Atlantic or Gulf of Mexico-based coastal EISs that mentioned OA, only three were land-based coastal EISs, one in South Carolina, one in Florida and one in New Hampshire (the rest were BOEM or NMFS EISs for off-shore drilling or fishing activities). The only states in which the impacts of OA on commercial fisheries or shellfish aquaculture were discussed were Alaska, Washington and California.

DISCUSSION

Burger and Wentz (2016) highlighted that the choice of how to account for GHG emissions from fossil fuels – at the point of emission or the point of fossil fuel extraction – has powerful implications for the assessment of GHG emissions under NEPA. Our results suggest that the choice of how to account for losses of ecosystem carbon storage and future sequestration also merit careful attention given their current treatment in NEPA processes.

Carbon Accounting for Cumulative Impacts

We have performed a quantitative assessment of the treatment of greenhouse gas emissions and carbon sequestration in ecosystems within hundreds of environmental impact statements from 2012-2015 in order to assess the status quo of carbon accounting practices within a salient environmental policy process. Within the

flexible policy space of draft federal guidance for greenhouse gas accounting in NEPA documents, our analysis reveals only a partial penetration of CEQ guidance into agency practice, as just under half of all coastal EISs included quantification of GHG emissions or any discussion of the relevant guidance on carbon accounting, and 20% did not discuss any impacts of climate change.

Despite the revised draft guidance in December 2014, highlighting the need to consider both the impacts from sources of GHG and the impacts of climate change's effects on ecosystems and to make such analysis proportional to the magnitude of the emissions, our results reveal that, even among EISs from 2015, there was limited quantification of emissions (55% of all EISs included GHG quantification) and 15% of coastal EISs in 2015 still did not include any discussion of any impacts of climate change. Thus, within the "flexible policy space" of draft guidance, uptake has been partial at best.

The unique challenge in considering GHGs under NEPA is in assessing the cumulative impacts of emissions (Kass 2009). Given that most human activities have a carbon footprint and that, unlike more conventional pollutants with local-scale impacts, each incremental ton of GHG emission causes proportional additional harm of global climate change, it is very easy to consider almost any amount of emission as globally insignificant, but very easy for the cumulative impacts of the emissions from multiple projects to rapidly become substantial. Indeed, our results show that even among the half of EISs for which GHG emissions were quantified, 5.9 million tons CO₂e, causing an estimated damage of over \$200 million, were emitted under

proposed action alternatives for which GHG emissions were found to have insignificant environmental effects.

Additionally, analyses of cumulative impacts must be able to consider multiple simultaneous stressors to the functioning of an ecosystem and from marine ecosystems (Halpern et al. 2008), to public health impacts on communities (Solomon et al. 2016), and there have also been recent calls for new integrated environmental management approaches that take into account the cumulative impacts of multiple stressors, including climate change. NEPA is one of the few environmental statutes in which multiple environmental issues are simultaneously considered, as opposed to the management of single species or single category of pollutant. As new advances in the modeling of the effects of climate change in addition to local stressors advances (Gillingham et al. 2016), reform of NEPA guidance around the consideration of the cumulative impacts of climate change provides an opportunity to engage in a form of adaptive management in which new methods for modeling and accounting are deployed.

The Little NEPA Effect

The majority of the proposed actions which included a quantification of GHG emissions were either energy projects or military operations. The only exceptions to this trend were found in California and Washington state, in which GHG emissions were quantified for a majority of non-energy and non-military proposed actions. Both California and Washington are among the sixteen states that have “Little NEPAs”, state statutes that include environmental impact reporting requirements. In California,

the California Environmental Quality Act (CEQA) includes mandatory reporting requirements for GHGs, and Washington has recently issued draft guidance for the consideration of GHG emissions under its State Environmental Policy Act (SEPA). The other coastal states with “Little NEPAs” include Connecticut, New York, New Jersey, Massachusetts, Maryland and Hawaii, none of which explicitly require consideration of GHG accounting. Additionally, management districts in California have begun to set thresholds for GHG emissions as air pollutants.

Across the United States, California and Washington are at the forefront of state climate policy development. California has an economy-wide cap and trade program, and Washington’s Governor Jay Inslee recently issued an executive order to create a regulatory cap on GHG emissions in Washington. Thus, state guidance on GHGs for little NEPAs and the institutional context of state-level climate policy action appears to affect the wider uptake of the practice of GHG accounting, and GHG quantification appears to be a function of the state policy-context.

The Air Quality Effect

Nearly without exception, those coastal EISs that reported quantified greenhouse gas emissions did so in units of carbon dioxide equivalent alongside the emissions of other conventional air pollutants, such as nitrogen oxides, particulate matter, and carbon monoxide, which have long been regulated under the Clean Air Act. These GHG emissions inventories were most frequently included under impact sections entitled “Air Quality.” This stands in stark contrast to the (more frequent) specific consideration of carbon storage and sequestration in forested ecosystems

within contemporaneous non-coastal EISs for forest management projects from the US Forest Service. For these EISs, carbon sequestration was not included under “Air Quality” sections but more frequently under “Forest Carbon Cycling and Storage” sections, within a more general “Affected Environment” section, thus being decoupled from any consideration of other air pollutants.

Carbon of Convenience

One of the consequences of largely considering GHG emissions as air pollutants is the widespread use of what we term “qualitative offsetting”. Ecosystem carbon sequestration, when considered alongside reports of GHG emissions from construction activities or fossil fuel combustion within a section on “Air Quality”, is considered a “mitigation measure” that reduces the impacts of the other emissions. Thus, ecosystem carbon storage, and in particular non-forest ecosystem carbon that does not have a robust developed methodology for accounting in which it is treated as its own impact, can be understood as a form of “carbon of convenience.” Non-forest ecosystems, such as coastal wetland ecosystems are only brought into the analysis as mitigation measures for fossil fuel combustion emissions. Their losses are not independently considered as sources of emissions, and when such losses are quantified it is only done so when net carbon sinks from restored ecosystems are also being considered.

Van Kooten (2009) argues that the unique attributes of biological carbon sequestration present challenges to simple quantification and measurement and highlights the rapidly accumulating potential transaction costs associated with

monitoring this carbon. One consequence of this challenge may be that ecosystem carbon sequestration accounting can be “hand-wavy”; we know ecosystems store carbon and that is a net benefit, but it is hard to know exactly how much. The costs associated with carbon monitoring were frequently highlighted in coastal EISs, and the net result of this circumstance appears to result in the kinds of implicit offsetting that we observed.

The conceptualization of GHGs as air pollutants coming from the combustion of fossil fuels can also lead to extreme conclusions that ignore the science of anthropogenic drivers of climate change. For example, in one EIS from South Carolina, GHG emissions from land management were not considered to be anthropogenic GHG emissions, despite the loss of carbon sequestration:

Unlike fossil fuels, such as natural gas and fuel oil, CO₂ emitted from prescribed burning [of coastal lands] is generally not counted as a GHG because it is considered part of the short-term CO₂ cycle since it does not introduce any new carbon that did not come directly from the atmosphere.

Writing before the CEQ published any draft guidance, Stein (2010) cautioned that, under the existing way NEPA is structured, consideration of climate change is likely not to be meaningful, and called for specific guidance on how to consider climate change impacts (Stein, 2010). Yet in our analysis of NEPA documents produced after the first draft guidance was released, we find that further specific guidance on the treatment of ecosystem carbon sequestration is needed to avoid such carbon being used selectively.

Blue Carbon

Despite the 2014 Revised Draft Guidance which specifically noted that emissions from the destruction of coastal wetlands should be considered, only 4 of 45 coastal EISs produced after this guidance was released mentioned carbon sequestration in coastal ecosystems, despite all proposed actions taking place in ecosystems with the potential to impact coastal wetlands. In addition to revealing that the status quo treatment of coastal carbon sequestration in EISs conceives of such carbon as a potential offset for project construction emissions, our results also highlighted several other aspects of the current conceptualization of blue carbon within federal agency thinking. The geographic distribution of blue carbon consideration in EISs was highly skewed, with the majority of consideration in the Pacific, Gulf of Mexico and New England. This may have been driven because particular blue carbon ecosystems are being more frequently considered as carbon sinks within EISs. Of the 21 coastal EISs that mentioned carbon sequestration in coastal ecosystems, 18 discussed carbon sequestration in salt marshes or coastally inundated peat soils, 5 mentioned carbon sequestration in sea grass meadows, 4 in mangrove forests, 2 in tidal flats, and 1 in kelp forests. Our data are not sufficient to determine if this distribution shows that salt marshes are being “normed” as a “carbon sink ecosystem” at a faster rate than others, or if it is a reflection of the higher rates of considering carbon sequestration in the policy geographies where salt marsh ecosystems occur (in our dataset, in San Francisco Bay and the Northeast).

Ecosystem Services

EISs must also consider cultural, social and economic impacts of proposed federal actions that are likely to have significant environmental impacts. In other words, the ecosystem services framework is well-aligned with the requirements of NEPA impact reporting. Our data reveal rapidly increasing, nearly exponential uptake of the ecosystem services framework within coastal EISs from 2012 to 2015. This increase is part of a wider trend in the increased attention of this framework in federal environmental decision-making, which culminated in an October 2015 White House Memo directing agencies to incorporate the values of ecosystem services into federal decision-making (Donovan et al. 2015). Current practice, however, indicates that quantified valuation of ecosystem services is not common practice and remains a challenging exercise within the NEPA governance context. What effect the new regulatory initiative and new best management practices and guidelines for ecosystem service valuation have on NEPA practice is an open question that should be addressed.

Our results also show that the use of the ecosystem services framework is often selective rather than comprehensive. No EIS in our dataset used the ecosystem services framework equally for all environmental effects or potential ecological consequences that were considered, and no EIS included a systematic description of the full set of ecosystem services provided, let alone any quantification. Rather, the framework was used in relation to specific examples of ecosystems, when the impacts of a proposed action or action alternative to that ecosystem were considered. For example, when impacts to wetlands were being considered, some of the services that coastal wetlands provide might be listed. Thus, while there are calls for ecosystem services to be used as an integrating framework as the basis for environmental

management (Granek et al. 2010), and this may be the ultimate effect of the White House's initiative, current agency thinking, as revealed within environmental impact statements, does not reflect such an integrated strategy. Frequently, observation scientists have voiced concerns that the widespread use of ecosystem services framework in decision-making will turn environmental management into an optimization problem that ignores specific species and favors easy-to-quantify ecosystem services like carbon storage, over other values generated by local wild places (Redford and Adams 2009). Our assessment of the current usage of ecosystem services within this decision-making context, suggests that this is not (yet) occurring.

Ocean and Coastal Acidification

While there has been growing attention to ocean acidification and its current and significant ecological and economic impacts in the United States (Cooley and Doney 2009; Eckstrom et al. 2015), including the creation of a NOAA Ocean Acidification Program through a congressional Act, the consideration of OA impacts in NEPA processes is currently minimal, and lags far behind the consideration of climate change in its penetration. It is difficult to imagine a coastal or marine ecosystem in which the potential impacts of OA on at least one species or ecosystem component do not at least bear some consideration in a discussion of the total environmental impacts of a proposed action, yet three-quarters of coastal EISs from 2012-2015 did not include these considerations.

Furthermore, despite recent, high-profile and extensive scientific literature focused on the local-scale drivers and impacts of coastally-enhanced acidification,

including studies directed specifically at environmental management professionals (Salisbury et al. 2008, Cai et al. 2011, Kelly et al. 2011; Kelly and Caldwell 2013; Strong et al. 2014, Gledhill et al. 2015, Mathis et al. 2015), only two coastal EIS documents included any discussion of localized drivers of coastally-enhanced acidification. Furthermore, despite numerous public comments on draft EISs highlighting the potential of local mitigation and OA remediation measures, only two EISs included any discussion of local phenomena that might mitigate OA and preserve carbonate concentrations. Coastal acidification and the potential for local mitigation of OA impacts is not currently normed within the professional environmental management community.

Overall, the results of our analysis indicate that discussion of ocean acidification impacts has not been mainstreamed, and that OA, if it is considered at all, is considered as a global phenomenon.

If OA is considered in a coastal EIS, it is primarily described as having impacts on corals. The exceptions to this were EIS documents from Alaska and Washington state, which included much more detailed discussion of the potential impacts of OA on commercial groundfish and shellfish fisheries and aquaculture. In part, this may be because these states' fishery and aquaculture industries are more immediately threatened by the impacts of OA due to the temperature of their waters and coastal upwelling, but Alaska and Washington state are also among the few coastal states that have initiated state-level processes to combat ocean acidification (Strong et al. 2014). Given the example of OA, EISs appear to reflect the state of the cycles of public discussion and concern, more than the state of emergent scientific knowledge, in their

consideration of impacts. And while the science of OA impacts is perhaps not as fully developed as the science of the impacts of climate change, it is clear that the production of 2010 Draft CEQ guidance has led to greater penetration of the consideration and reporting of GHG emissions (as evidenced by the frequent reference to it alongside GHG quantification in EIS statements), even in the absence of a finalization of the guidance. Given the current status quo of consideration of ocean acidification impacts, and given that OA impacts not mentioned in either the 2010 Draft Guidance or the 2014 Revised Draft Guidance, there is salient need for specific guidance from CEQ on the consideration of OA impacts, drivers and mitigation measures within NEPA processes.

CONCLUSIONS

We have assessed the current state of the consideration of impacts to blue carbon, ocean acidification impacts, and other coastal ecosystem services within coastal environmental impact statements. Each of these salient and emergent environmental problems and frameworks exhibits some initial and partial uptake within the existing institutional framework of the National Environmental Policy Act. What we do find to be current practice is the recognition of the carbon sequestration values of some coastal ecosystems – especially salt marshes– the recognition of the current and potential impacts of global OA, and a recognition of the existence of non-market ecosystem service values provided by coastal ecosystems as a framework for reporting impacts. In particular, we find that ecosystem carbon sequestration is most frequently considered when this service is enhanced through restoration projects.

What is absent is any meaningful recognition of the impact of lost coastal ecosystem carbon sequestration as a result of proposed construction, dredging or other destructive activities. This stands in contrast to the treatment of carbon sequestration in forests within US Forest Service EISs, which report on the potential impact of carbon storage lost by harvest or fire. If further development and policy guidance on accounting for GHG impacts is contemplated, it must take as a starting point the status quo of how these emergent problems are currently being conceived.

Taken as a whole, our results show that the tailored policy process in the United States, which treats GHG as air pollutants, coupled with the more rapid emergence of detailed accounting rules for GHG emissions from fossil fuel combustion than for anthropogenic impacts on natural lands and ecosystems, has led to a circumstance where ecosystem carbon sequestration is currently regarded as carbon of convenience. Outside of forests, ecosystem carbon appears only to be discussed when proposed actions augment carbon stocks and thereby mitigate other GHG emissions from fossil fuel combustion. Combating the propensity to only consider ecosystem carbon storage when it is convenient is critical to the scientific integrity and robustness of climate policy development, and we suggest that further CEQ guidance should focus on how best to include emissions from the destruction of coastal ecosystems.

As our understanding of the modalities of adaptive management increases, our results highlight the strong influence of institutional context in shaping the specific form of uptake of new scientific knowledge. The local and state policy-contexts for the consideration of greenhouse gases, in addition to agency institutional norms,

strongly shaped the ways in which coastal carbon cycling was incorporated into this federal policy process. As we continue to advance our understanding of how human activities influence ecological functions, it is important to recognize that the successful management of those influences is as shaped by the governance structures that are used to manage them as by the scientific knowledge of the impacts.

Chapter 5

The response of San Francisco Bay-Delta phytoplankton to ammonium, nitrate, and wastewater effluent additions under different light conditions⁷

ABSTRACT

Since the 1980s, the San Francisco Bay-Delta (SFBD) ecosystem has experienced large declines in primary production. Hypothesized reasons for this decline include light limitation of production, the rapid invasion of the ecosystem by the filter-feeding clam species *Potamocorbula amurensis* (formerly *Corbula amurensis*), the suppression of nitrate (NO_3^-) uptake and thus phytoplankton growth due to high concentrations of anthropogenic ammonium (NH_4^+) from wastewater effluent, and wastewater NH_4^+ -induced changes in phytoplankton community composition away from large-celled diatoms. Here, we present results from a 48-hour incubation experiment with surface water from both upstream and downstream from the Sacramento Regional Wastewater Treatment Plant effluent outfall, a major source of NH_4^+ loading to the ecosystem. We amended this water with NH_4^+ , NO_3^- , or a full wastewater effluent addition. All sample waters were incubated under high light (52% irradiance) or low light (6% irradiance). NO_3^- uptake rates were suppressed to near zero in all treatments with added NH_4^+ , added wastewater effluent, or high in situ NH_4^+ concentrations. Yet, phytoplankton uniformly grew well on all sources of DIN,

⁷ A version of this chapter is currently under review at *Limnology and Oceanography*. It was written with Matt Mills, Ivy Huang, Sara Thomas, Mine Berg, Raphael Kudela, Stephen Monismith, Chris Francis and Kevin Arrigo.

including effluent and clean NH_4^+ . Diatom species were the most abundant taxa in all stations and diatom cell abundances increased at greater rates than all other taxa over the course of the experiment. Among all treatments, the light treatment had the greatest effects on Chl *a* accumulation and phytoplankton growth. These results suggest that high anthropogenic NH_4^+ loading to the SFBD is not a driver of the lower productivity in the SFBD.

INTRODUCTION

Globally, increases in fixed nitrogen (N) delivery to ecosystems from anthropogenic sources risk fundamentally altering the functioning of the earth system (Rockstrom et al. 2009) and are a salient indicator of the Anthropocene (Steffen et al. 2015). Human activities, from agricultural production to wastewater management, increase loading of anthropogenic dissolved inorganic N (DIN) to aquatic ecosystems, leading to eutrophication, hypoxia, biodiversity loss, toxic drinking water supplies, harmful algal blooms and coastal acidification (Galloway et al. 2008, Howarth 2008, Fowler et al. 2013, Strong et al. 2014). Nearly all of these problems are linked to the response of aquatic phytoplankton communities to excess nutrient loading, and the management of DIN loading is a central focus of water quality regulations, both in the United States and globally (Krenkel 2012; Davies and Mazurek 2014).

N exists in aquatic ecosystems in multiple chemical states, including organic N, ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-). Both NO_3^- containing fertilizers, which are commonly used in agricultural production, and terrestrial nitrification, the microbial conversion of NH_4^+ to NO_3^- , result in agriculture being a major source of

$\text{NO}_3\text{-N}$ to coastal aquatic ecosystems. In contrast, NH_4^+ is delivered to coastal waters via atmospheric deposition and wastewater discharge. In fact, agencies tasked with permitting discharges from wastewater treatment plants (WTPs) face decisions over whether to permit discharge N as organic N, NH_4^+ , or NO_3^- following a nitrification treatment. Alternatively, WTPs can implement both nitrification and denitrification and release N as dinitrogen (N_2) gas (Wunderlin et al. 2012).

The transformation of N species between chemical forms is a principal focus of biogeochemical, civil engineering, and microbial ecological research on the N cycle (Carpenter and Capone 2013; Casciotti and Buchwald 2015). Many problems that stem from anthropogenic DIN loading are the result of the multiple forms of DIN that are released and their differential effects on phytoplankton ecology. Given this, our understanding of how specific forms of introduced DIN impact phytoplankton productivity and community composition in human-impacted aquatic ecosystems remains surprisingly uncertain, and is an area of active research (Berg et al. 2003, Heil et al. 2007, Esparza et al. 2014; Glibert et al. 2016).

NH_4^+ is the most reduced form of dissolved N and requires the least amount of energy by phytoplankton to assimilate and utilize in the synthesis of amino acids. In contrast, NO_3^- must be reduced to ammonia (NH_3) through a series of reductive steps, starting with the enzyme nitrate reductase, prior to being incorporated into amino acids (Dortch 1990). This difference in redox state between NH_4^+ and NO_3^- would suggest that phytoplankton cells grown under replete NH_4^+ and NO_3^- conditions should preferentially utilize NH_4^+ . Thus, in the presence of a biochemically meaningful concentration of NH_4^+ , the rate of NO_3^- uptake by phytoplankton cells is expected to

be reduced until the supply of NH_4^+ is exhausted. This is frequently the case in aquatic ecosystems (Ludwig 1938; Harvey 1953).

This apparent suppression of NO_3^- uptake in the presence of NH_4^+ has been repeatedly demonstrated in multiple phytoplankton taxa such as diatoms (Cresswell et al. 1979, Hildebrand and Dahlin 2000, Song and Ward 2007), chlorophytes (Syrett 1988, He et al. 2004), cyanobacteria (Ohmori et al. 1977), and haptophytes (Song and Ward 2007). Due to the lower amount of energy required for assimilation, some diatom species are able to maintain a faster growth rate when grown on NH_4^+ relative to other DIN forms (Thompson et al. 1989; Suksomjit et al. 2009; Tada et al. 2009). Evidence for NH_4^+ inhibition of NO_3^- uptake by phytoplankton in situ has also been observed in aquatic estuarine environments, usually when NH_4^+ concentrations are above 2 μM , such as in the Delaware Bay (Pennock 1987), the Neuse River (Boyer et al. 1994), Monterey Bay (Berges et al. 1995), and San Francisco Bay (Dugdale et al. 2007)

In coastal ecosystems impacted by anthropogenic DIN loading, both NH_4^+ and NO_3^- may be present in high concentrations. In such ecosystems, total annual DIN uptake can be dominated by NH_4^+ (Pennock 1987; Boyer 1994; Kocum et al. 2002). In some of these systems, NO_3^- uptake rates were found only to increase during blooms once NH_4^+ sources were depleted. Such observations raise a key ecological, rather than biochemical, question: Under N-replete conditions, does the speciation of DIN being taken up affect the over-all primary productivity of an estuarine ecosystem? That is, for a given total anthropogenic DIN load, will net phytoplankton production

be the same regardless of whether the DIN is in reduced (NH_4^+) or oxidized (NO_3^-) form?

A second ecological question is: how is phytoplankton community composition affected by the form of available DIN? Aquatic phytoplankton ecologists have long observed that large-celled species tend to be more competitive in aquatic environments with high concentrations of DIN as NO_3^- and small-celled species are more competitive in low nitrogen regions (Chisholm 1992). The species in upwelling and oligotrophic environments differ markedly in their cellular N requirements and utilization strategies. Upwelling ecosystems, with a steady supply of NO_3^- , tend to be dominated by diatoms, while the oligotrophic gyres favor the growth of cyanobacterial picophytoplankton such as *Prochlorococcus* and *Synechococcus*. Because aquatic ecosystems with both naturally high ambient NO_3^- and NH_4^+ concentrations are rare (due to phytoplankton consumption of these nutrients), the composition and physiology of phytoplankton communities that favor these environments are not clearly understood.

The above two questions are fundamental to our understanding of aquatic ecosystem functioning, to our understanding of the services such ecosystems deliver, and to science-based environmental decision-making. In particular, in the United States such questions are relevant to the choice of form of N loading from wastewater treatment plants faced by water quality managers implementing Clean Water Act provisions (Carey et al. 2012). These questions are particularly salient in the highly impacted San Francisco Bay/Delta ecosystem.

San Francisco Bay-Delta (SFBD)

San Francisco Bay and the Sacramento-San Joaquin River Delta form the largest estuary on the western coast of North America, designated here as the San Francisco Bay/Delta (SFBD) (Figure 5-1). The Sacramento River drains the northern portion of California's Central Valley. The city of Sacramento is situated at the confluence of the Sacramento River and the American River, which drains the Sierra Foothills. From Sacramento, the Sacramento River flows south, joining several in-Delta slough networks and a now unused shipping channel just upstream of the city of Rio Vista, CA. In this study, we refer to this area as the "Cache Slough Complex". From here, the Sacramento River enters northern San Francisco Bay, which is also fed by the San Joaquin River, which itself drains the southern portion of California's Central Valley. Northern San Francisco Bay itself is made up of several sub-embayments, including Suisun Bay, Grizzly Bay, and Honkers Bay. These waters exchange through the Carquinez Strait with San Pablo Bay and Central Bay, which opens to the Pacific Ocean via the Golden Gate entrance.

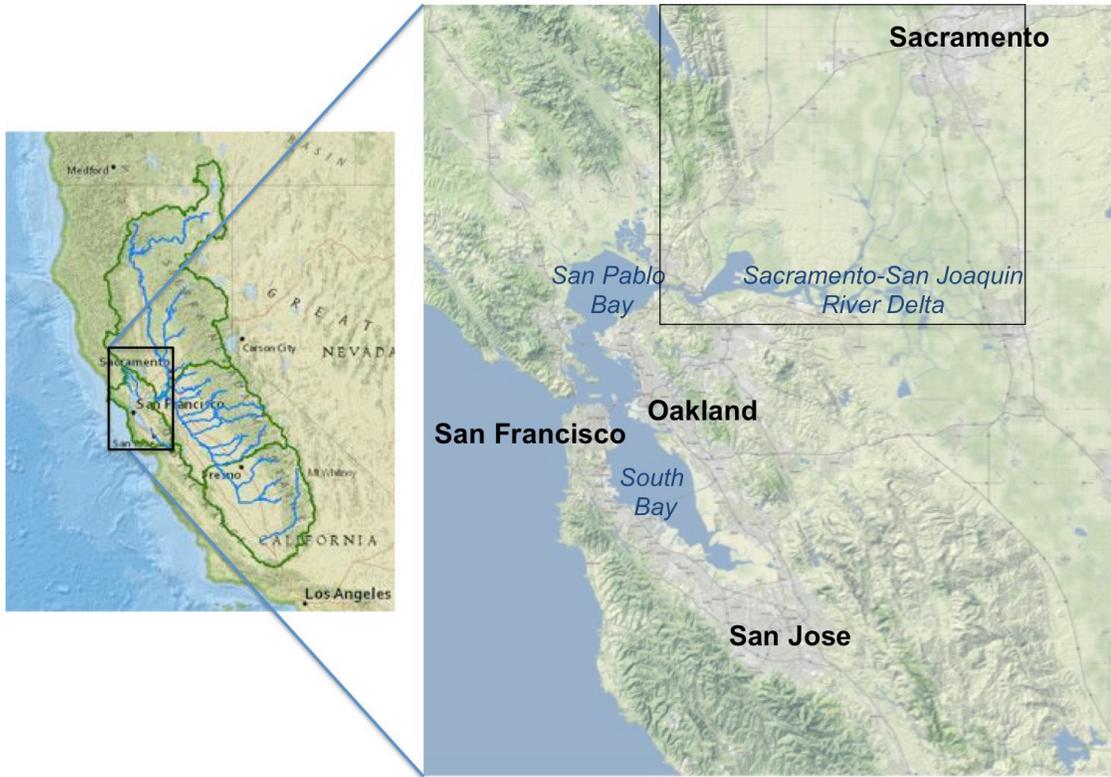


Figure 5-1. The San Francisco Bay Estuary, showing the location of the Sacramento San Joaquin River Delta.

The northern portions of SFBD have experienced significant ecological changes over the past several decades (Cloern and Jassby 2012). Most notably, phytoplankton production in Suisun Bay and the Delta dropped dramatically beginning in 1986 (Jassby et al. 2002). The decrease in productivity has cascaded up the food web, affecting the productivity of higher trophic levels, including copepod and mysid shrimp species (Kimmerer 2006), leading to an overall decline in fish production throughout the northern SFBD (Sommer et al. 2007). This phenomenon is known as pelagic organism decline (POD), and has been a major focus of federal and state agency management efforts in the Bay Delta region for decades. In particular, one species suffering under POD is the Delta smelt (*Hypomesus transpacificus*), a federally listed species under the Endangered Species Act of 1973.

The POD presents a paradoxical puzzle, because the SFBD ecosystem is a high nutrient environment, receiving large inputs of anthropogenic N from Central Valley agricultural runoff (as NO_3^-), and from wastewater treatment plant discharges (as NH_4^+). In particular, the Sacramento Regional Wastewater Treatment Plant (SRWTP) effluent outfall located downstream of the City of Sacramento discharges approximately 15 metric tons of N- NH_3 per day (Senn and Novick 2014) into the lower Sacramento River, and NH_4^+ concentrations downstream of the effluent discharge are frequently 50-100 μM . In Suisun Bay, which receives water from both the Sacramento and San Joaquin Rivers, NO_3^- and NH_4^+ concentrations regularly exceed 50 and 5 μM , respectively. The corresponding increase in phytoplankton biomass normally associated with high DIN inputs is not observed in the SFBD. Thus, whereas managers of many human-impacted estuarine systems around the world are primarily concerned about excessive algal growth due to high anthropogenic N loading leading to eutrophication and hypoxia (Howarth 2008), environmental managers in the SFBD are concerned about the decreased phytoplankton productivity and POD despite high concentrations of anthropogenic N (Novick et al. 2014).

Several alternative explanations for POD have been advanced. First, the decline in productivity in Suisun Bay is highly correlated to the invasion of the SFBD by the over-bite clam, *Potamocorbula amurensis*, which was first introduced and became established in 1986 (Jassby 2008; Cloern and Jassby 2012). The grazing rate of these benthic organisms can be greater than the rate of primary production (Werner and Hollibaugh 1993; Greene et al. 2011), thereby preventing phytoplankton bloom formation in Suisun Bay and other regions where the clam is present (Thompson

2005). In South San Francisco Bay, Thompson et al. (2008) found that both benthic grazing and wind-driven turbidity in shallow waters controlled the formation of phytoplankton blooms. However, this invasive clam species has only just begun advancing from Suisun Bay up the lower Sacramento River, a region that has also experienced low phytoplankton productivity (Jassby 2008). The Sacramento River and the Delta are dominated by another clam species, the freshwater *Corbicula fluminea* (Lucas et al 1998), which also exhibits high grazing rates (Lopez et al. 2006).

A second potential driver of lower phytoplankton production is light limitation. Alpine and Cloern (1992) showed that high suspended particulate matter (SPM) concentrations in Suisun Bay were sufficient to suppress photosynthesis by phytoplankton. Light limitation has been identified as a powerful control of phytoplankton productivity throughout SFBD and in many other turbid estuaries (Cloern 1987), yet changes in the light environment in the SFBD are not closely correlated with the timing of the sharp drop in phytoplankton productivity in the northern SFBD that started in the mid-1980s. In fact, the reverse trend in suspended particulate matter (SPM) has been the norm over the last several decades. The turbidity of the SFBD has been declining, owing to the gradual flushing/settling of sediments moved by 19th century mining activities (Wright and Schoellhammer 2004). The changes in sediment delivery have actually resulted in a deepening of the photic zone. Cloern and Jassby (2012) highlight that Suisun Bay photic depths have deepened from 1.3 to 2.0 m due to recent reductions in sediment supply. Thus, in the SFBD, light availability is not obviously linked to the shift in productivity associated with POD. Phytoplankton bloom dynamics are also closely linked to fresh water flow

regime, which controls stratification and residence time in the SFBD. With greater freshwater flow, density-driven stratification can prevent mixing and increase light availability in surface waters, allowing the formation of blooms (Lucas et al. 1998; Jones et al. 2009).

Finally, a recent body of experimental work has suggested that the high concentration of NH_4^+ from wastewater effluent from the Sacramento Regional Wastewater Treatment Plant (SRWTP) may be linked to POD. Glibert (2010) specifically implicated changes in DIN nutrient loading and DIN speciation to POD, via the effect of high NH_4^+ concentrations on phytoplankton growth. Broadly, the mechanistic arguments of the “ammonium” hypothesis are two-fold. First, Dugdale et al. (2007) argued that concentrations of $\text{NH}_4^+ > \sim 4 \mu\text{M}$ inhibit the use of NO_3^- by phytoplankton cells, and that due to slower overall growth rates on NH_4^+ than NO_3^- , high NH_4^+ loading results in depressed total productivity and the prevention of bloom formation. This is supported by Parker et al. (2012) who found that specific uptake rates (V_{max}) for NO_3^- were greater than for NH_4^+ , and that growth on NH_4^+ suppressed carbon fixation by phytoplankton in the lower Sacramento River, lowering overall productivity. Second, high concentrations of NH_4^+ from wastewater effluent supposedly induced a shift in the Bay Delta phytoplankton community. Wilkerson et al. (2006) argued that during spring phytoplankton blooms, higher NH_4^+ concentrations favor the growth of non-diatom species such as cryptophytes and chlorophytes, and Glibert et al. (2014) argue that the decline in productivity in the Bay Delta is a function of the transition away from a diatom-dominated community.

Fundamentally, the SFBD is a highly-impacted, critical resource to the state of California. Understanding the impacts of high anthropogenic NH_4^+ loading from wastewater on phytoplankton productivity and community composition in this system is critical to addressing questions about the contributions, if any, of anthropogenic NH_4^+ loading to the POD, as well as to fundamental questions of phytoplankton ecology in human-impacted ecosystems. It is also critical to addressing questions faced by water quality decision-makers in a region that faces a broad suite of global change threats ranging from drought to sea-level rise.

Here, we contribute to the understanding of the causes of low phytoplankton production in the SFBD by presenting results from a replicated set of experimental incubations of in situ phytoplankton communities from the lower Sacramento River, both upstream and downstream of the SRWTP discharge site.

METHODS

In order to assess the effects of DIN species and light on phytoplankton productivity and community composition, we incubated samples at two different light levels after adding either NH_4^+ , NO_3^- , or final wastewater effluent (hereafter “effluent”) to water from two stations upstream from the SRWTP outfall pipe. In conjunction with the experiment, we conducted a detailed river surface sampling transect in the lower Sacramento River where we measured chlorophyll *a* (Chl *a*) fluorescence, the maximum efficiency of photosystem II (Fv/Fm), temperature, salinity, and turbidity. We also made high-frequency measurements of surface NH_4^+ and NO_3^- concentrations. Through the combination of experimental incubation of

phytoplankton under various nutrient and light regimes and high-frequency DIN sampling, we were able to assess the roles of wastewater NH_4^+ loading and light limitation in driving phytoplankton productivity and shifts in phytoplankton community composition in this impacted aquatic ecosystem.

Sample water collection

Near-surface water was collected from the R/V *Questuary* between 0730 and 1130 on 6 May 2015 from three locations in the Sacramento River (Figure 5-2). Station A (the Interstate Highway 80 Bridge) was located immediately upstream of the confluence of the American and Sacramento Rivers at 38.5997°N and 121.5513°W (Figure 5-2). Station B (upstream of effluent outfall) was located at 38.5163°N and 121.5455°W downstream of the confluence of the American and Sacramento Rivers, but upstream of the SRWTP effluent outfall pipe. Station C (downstream of effluent outfall) was located at 38.4348°N and 121.5163°W. Station B is close to USGS- Station 29: Garcia Bend, and Station C is near USGS- Station 26: River Mile 44.

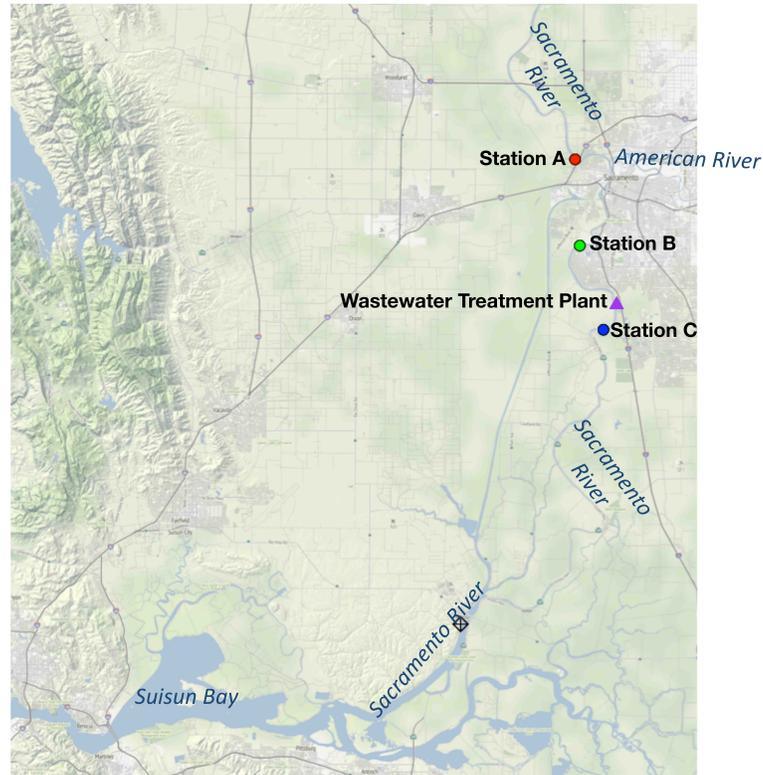


Figure 5-2 Three station locations are shown. Station A was located upstream from the confluence of the American and Sacramento Rivers and upstream of the WWTP effluent outfall. Station B was located downstream from the confluence of the American and Sacramento Rivers and upstream of the WWTP effluent outfall. Station C was located downstream of the WWTP effluent outfall.

At all stations, surface water was collected using pre-washed plastic (HDPE) 20 L buckets deployed by hand from the ship deck. Water was poured through a funnel equipped with a 300 μm Nitex screen filter into 10 L acid-washed (10% HCl) cubitainers (ThermoScientific™ LDPE Poly-Cubitainers) to remove macrozooplankton and large detrital particles. At Stations A and B, 24 10 L cubitainers were filled and at Station C, 6 10 L cubitainers were filled. All cubitainers were placed in the dark during transit to the experimental location at the Delta Marina in Rio Vista, CA (38.1491°N, 121.6925°W).

Water column profiles and sampling

At each water sampling station, two water column profiles were obtained using the ship-board SBE-19 CTD with ancillary sensors (Sea-Bird Electronics, Bellevue, Washington 98005 USA). Vertical profiles of salinity, temperature, depth, turbidity (via optical backscatter, OBS), Chl *a* fluorescence, and photosynthetically active radiation (PAR) were measured. The CTD recorded measurements at 0.5 Hz in both the down and up-cast profile. Two liter Niskin bottles were fired from a SBE32 water carousel to collect water at near-surface (1 m below surface), mid (5 m below surface) and near-bottom (~10 m below surface) depths.

Under-way measurements

The day prior to sample collection, we conducted a river transect headed upstream in the Sacramento River from the Delta Marina in Rio Vista, CA (38.1491°N, 121.6925°W) to the River Bank Marina in Sacramento (38.6034°N 121.5164°W). During this transect, under-way measurements were made of surface salinity, temperature, Chl *a* fluorescence, variable fluorescence (Fv/Fm), and NO₃⁻ and NH₄⁺ concentrations. On the day of sample collection, these same underway measurements were made while transiting between stations A, B, and C.

Briefly, while under-way, surface water was continuously pumped through an open-bucket chamber on deck, outfitted with conductivity, temperature, Chl *a* fluorescence and optical backscatter sensors (Yellow Springs Instruments, Ohio). Surface water was also pumped in-line past an optical variable fluorescence sensor blue-light PhytoFlash (Turner Designs, San Jose, CA., USA), which measures Fv/Fm

of phytoplankton present in surface waters, logged at 30 s intervals. Surface water was also pumped through an in-line In-Situ Ultraviolet Spectrophotometer (ISUS) Optical NO_3^- sensor (ISUS, Satlantic Inc, Halifax, NS), which measured surface NO_3^- concentrations at 1 s intervals, with a detection limit of $2\mu\text{M NO}_3^-$.

Surface water from the flow-through open-bucket chamber was also sampled using a custom-designed, field-deployable flow-injection analysis-gas-diffusion (FIA-GD) based NH_4^+ sensor (FIALab 1500, Bellevue, WA), adapted from the wet-chemical method of Holmes et al. (1999) that measures fluorescence after reaction of NH_3 with ortho-phthaldialdehyde (OPA). Water was pumped from the chamber continuously through a 0.32cm ID tube using a peristaltic pump. Sample water was mixed with a carrier (de-ionized H_2O) and 20 mM NaOH to increase pH, converting all NH_4^+ in the sample water to NH_3 . The water was then passed by a gas-permeable membrane, which allowed any NH_3 present to diffuse into a stream of fluorometric development reagent (OPA, sodium tetraborate decahydrate, sodium sulfite). The reagent- NH_3 mixture was pumped around a heated (65°C) coil to increase the rate of development and measured fluorometrically. The frequency of measurements is related to how well discrete injections are resolved fluorometrically. More frequent sample injections raise the detection limit, while less frequent sample injections decrease the total amount of data collected. Our choice of injection rate gave a detection limit of $0.3\mu\text{M NH}_4^+$ with measurements made every 105 s while under-way.

Incubation experiment design and sampling

From 15:00 on 6 May 2015 until 15:00 on 8 May 2015, 54 10 L cubitainers filled at Stations A, B, and C were incubated in two square, opaque white-plastic incubators placed on the dock at the Delta Marina in Rio Vista, CA. Each incubator was continuously supplied with ambient-temperature near-surface Sacramento River water.

Nutrient amendment treatments

Our experiment comprised a total of nine combinations of sampling water and nutrient additions, as shown in Figure 3. Water from both Stations A and B (both upstream of the outfall) was kept as an un-amended control and treated with either NH_4^+ using clean laboratory grade NH_4Cl (Fisher Chemical, Pittsburgh, PA), NO_3^- from clean laboratory grade KNO_3 (Fisher Chemical, Pittsburgh, PA), or filtered (0.2 μm) effluent, obtained from the SRWTP the day prior to the experiments.

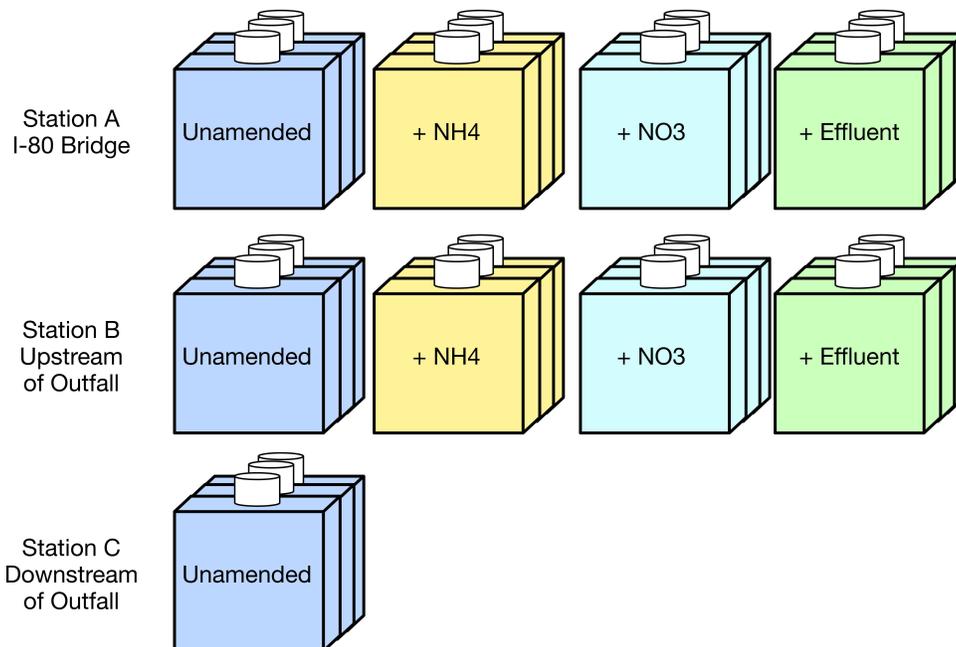


Figure 5-3. Experimental treatments and replicates for water from three stations.

Clean NH_4^+ and effluent additions were targeted to increase ammonium concentrations to $55 \mu\text{M NH}_4^+$, a concentration equivalent to that measured in the water column at Station C using the underway NH_4^+ analyzer (described above) on the day of sampling. Clean NO_3^- additions were targeted to add $7.5 \mu\text{M NO}_3^-$, a concentration equivalent to that measured in the water column at Station C using the underway NO_3^- analyzer on the day of sampling. All additions were made via autoclaved pipet. Water from Station C (downstream of the outfall) was also kept as an un-amended control and not subjected to any nutrient additions. Figure 5-3 shows the combination of nutrient treatments and replicates in the experiment.

Light treatments

Each of the two opaque white-plastic incubators housed 27 cubitainers, representing triplicate samples for all 9 combinations of station and nutrient treatments. Each incubator was then subjected to a different light level, using neutral density screening placed over the top of the incubator. The High Light (HL) treatment held cubitainers at 52% of ambient PAR and the Low Light (LL) treatment held cubitainers at 6% of ambient PAR. PAR was obtained from the average of multiple measurements (as % of PAR just below water surface) made prior to the start of the experiment within each incubator using a scalar PAR sensor (QSL-2100, Biospherical Instruments, San Diego, CA) inserted through a small hole in the side of the incubator. PAR measurements were corrected for attenuation by the plastic sides of the cubitainer.

Using PAR extinction profiles from the water columns at each of our three sampling locations, we calculated the water column depths for each location represented by the HL and LL light levels, as shown in Figure 5-4. To assess the performance of the light treatment throughout the duration of the 48 hour experiment, we placed a square cosine luminosity detector (HOBO™ Pendant, Onset Corporation, Bourne, MA) extended from a metal fastener into the middle of each incubator, and also placed two square cosine detectors outside exposed to ambient out-of-water irradiance.

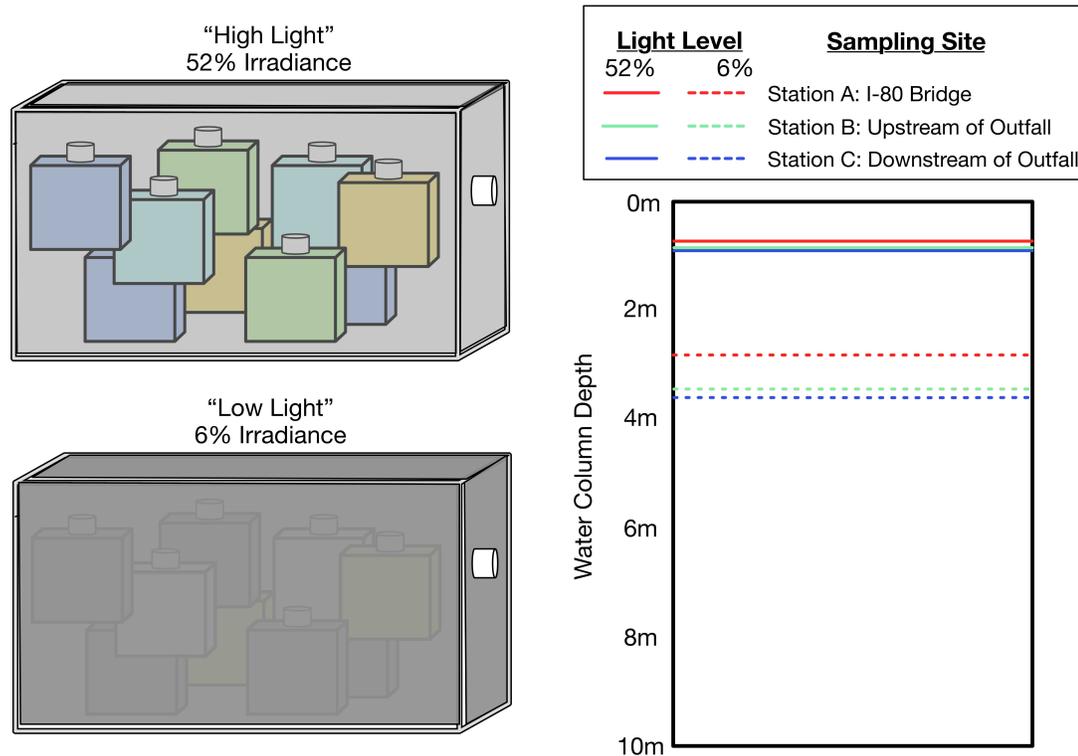


Figure 5-4 Light treatment levels and corresponding water column depths for each treatment at each station.

Chlorophyll a and phytoplankton physiology analyses

75 mL samples for fluorometric analysis of Chl *a* were filtered onto 25 mm Whatman glass fiber filters (GF/F, nominal pore size 0.7 μm), and placed in 5 mL of 90% acetone in borosilicate tubes. Chl *a* was extracted in the dark at 4°C for 12-24 hrs. Chl *a* was then measured fluorometrically (Holm-Hansen et al. 1965) using a Turner Fluorometer 10-AU (Turner Designs, Inc.). Chl *a* concentration was measured in triplicate from each sampling location prior to the start of the experiment. Samples for fluorometric analysis were then taken at all five time points. At $t=36$ h, the volume filtered was changed from 75 mL to 50 mL due to increasing Chl *a* concentrations.

At the three sampling locations, F_v/F_m was measured using a pulse amplitude modulation (WATER-PAM) fluorometer shipboard immediately following sample collection. Samples were collected in 50 mL tubes, dark-acclimated for 30 min at ambient temperature, and measured on a PAM (Heinz Walz GmbH, Effeltrich, Germany). The PAM was configured with the standard red (660 nm peak actinic illumination) WATER-ED unit, and blanked with filtered sample water.

At all five time points during the experiment, we also measured F_v/F_m and functional absorption cross section (σ_{PSII}) by fast repetition rate fluorometry (FRRF, LIFT-FRR, Soliense, Santa Cruz, CA) (Kolber et al. 1998). Samples were dark-acclimated for 30 min at ambient temperature, and measured on the FRRF within four hours of collection. Blanks for individual samples analyzed by FRRF were prepared by syringe filtration of sample water through a 0.2 μm polycarbonate filter. All F_v/F_m values were corrected for blank effects (Cullen and Davis, 2003). Several samples were measured on both PAM and FRRF to confirm their agreement.

Nutrient analyses

Samples were taken for nutrient analysis at the water collection stations and at each time point. Nutrient samples for NH_4^+ , $\text{NO}_3^- + \text{NO}_2^-$, and phosphate (PO_4^{3-}) were filtered through a 0.2 μm polycarbonate syringe filter (Acrodisc® Syringe Filter with Supor Membrane, Pall Laboratory, Port Washington, NY) into plastic collection vials and frozen at -20°C until analysis. Samples for dissolved silica (dSi) analysis were filtered through a 0.2 μm polycarbonate syringe filter and kept at 4°C until analysis.

NH_4^+ samples were analyzed by fluorometric development OPA on a FIALab 1500 ammonia analyzer. NO_3^- samples were analyzed colorimetrically using the cadmium-reduction method on a SmartChem 200 discrete analyzer (Unity Scientific, Brookfield, CT). PO_4^{3-} samples were analyzed colorimetrically using the ascorbic acid method on a spectrometer (Towns 1986). Dissolved silica (dSi) samples were analyzed on an Inductively Coupled Plasma Spectrometer (ThermoScientific ICAP 6300 Duo View). All analyses were performed using a standard curve at relevant concentrations and the maximum error for replicates was 5%. All nutrient analyses were performed in laboratories at Stanford University.

POC/PN

Particulate organic carbon (POC) and particulate nitrogen (PN) were analyzed by filtering sample water onto pre-combusted 25 mm Whatman GF/F filters. The filters were fumed with hydrochloric acid (HCl), dried at 60°C , and transferred to tin capsules (Costech Analytical Technologies, Inc.) for analysis on an Elementar Vario

EL Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) at the University of California, Davis.

¹⁵N uptake analyses

Measurements of NO₃⁻ and NH₄⁺ uptake rates were made from all treatment cubitainers every 12 hours during the experiment using the ¹⁵N stable isotope tracer method (Capone and Glibert, 1993). Briefly, two subsamples from each cubitainer were taken in clean 250 mL polycarbonate square bottles, one for NH₄⁺ uptake and one for NO₃⁻ uptake. To measure ¹⁵N uptake, bottles were spiked with clean-labeled nutrients (K¹⁵NO₃ or ¹⁵NH₄Cl) equivalent to 10% of the estimated concentration in the sample. Bottles were placed back into separate shaded incubators (HL=52% ambient irradiance and LL=6% ambient irradiance conditions) and incubated for 4 hours. After four hours of incubation, 125 mL was filtered onto a pre-combusted 25 mm Whatman GF/F. The δ¹⁵N of PN was measured following the above protocol for PN. Samples for analysis of NH₄⁺ and NO₃⁻ concentrations were also taken at the beginning and end of each 4 hour incubation. Specific uptake rates (V_{max}) for each DIN species were calculated following Dugdale and Goering (1967):

$$V_{\max} = ({}^{15}\text{N}_p - {}^{15}\text{N}_{\text{ue}}) / ({}^{15}\text{N}_{\text{diss}} - {}^{15}\text{N}_p * t)$$

where ¹⁵N_p is the ¹⁵N atom percentage (atom%) in the particulate fraction, ¹⁵N_{ue} is the measured ¹⁵N atom percentage in the unenriched sample, ¹⁵N_{diss} is the atom% ¹⁵N in the dissolved fraction after spiking, and t is the time in hours of the incubation. V_{max} is the N-specific uptake rate in units of hr⁻¹. The absolute rate of N uptake, ρ, in units

of $\mu\text{mol N L}^{-1} \text{ hr}^{-1}$, was calculated as the product of the N-specific rate and PN concentration (Dugdale and Goering 1967).

Phytoplankton enumeration

Sub-samples were taken in triplicate in 125 mL dark amber bottles from each of the three initial sampling locations (A, B, and C) prior to the start of the experiment (n=9) and fixed with 2% acid Lugol's Solution and kept in the dark until analysis.

Sub-samples were taken in 125 mL dark amber bottles at t=48 hours from all experimental cubitainers (triplicate measurements of each treatment at each light level, n=54), and fixed with 2% acid Lugol's Solution and kept in the dark until analysis.

For analysis, samples were filtered onto a 0.2 μm polycarbonate membrane (Nuclepore) and enumerated using a Leica DMLB compound microscope according to McNabb (1960) as described in Beaver et al. (2013). Briefly, at least 400 natural units (colonies, filaments, and unicells) were enumerated to the lowest possible taxonomic level from each sample. The abundance of common taxa was estimated by random field counts. Rare taxa were quantified by scanning a transect of the filter. In the case of rare, large taxa, half of the filter was scanned and counted at a lower magnification. Cell volumes (biovolumes) were estimated by applying the geometric shapes that most closely matched the cell shape (Hillebrand et al. 1999). Biovolume calculations were based on measurements of 10 organisms per taxon for each sample where possible. Mean biovolume values were computed for any sampling event that included duplicate samples. Phytoplankton microscopy was performed by BSA Environmental Services, Inc (Beachwood, OH, USA).

RESULTS

Under-way transects

Under-way transect data were obtained during up- and down-river transects on the day prior to and the day of water collection for experimental incubation. Surface water temperature in the lower Sacramento River on both transect days averaged $21.1 \pm 0.8^\circ\text{C}$. Over the course of the diel cycle, near-surface water temperatures in the lower Sacramento River increased from 19.5°C just before dawn to 22.5°C shortly after solar noon. Surface water temperature at both upstream sampling stations (A and B) was 20.1°C , whereas the water temperature was 20.6°C at Station C. There was a pronounced step-increase in surface water temperature of 0.5°C observed on both 05 May 2015 and 06 May 2015 from the effluent outfall. The sampling sites outside of the lower Sacramento River experienced strong tidal mixing and their water temperature were on average $1.5\text{-}2.0^\circ\text{C}$ cooler.

The region of the Sacramento River where we sampled was dominated by fresh water (salinity <0.2). At the far upstream end of our transect, upstream of the confluence of the American and Sacramento Rivers, salinity was 0.06, increasing downstream to 0.17 where the Sacramento River joins water from Suisun Bay and the San Joaquin River, in the Cache Slough Complex. Salinity at Rio Vista varied tidally and reached >2 during our sampling. Salinities at all three Stations (A, B, and C) were uniformly low (<0.1).

Surface water turbidity was nearly uniform throughout our sampling region in the lower Sacramento River, ranging by no more than ± 3 NTU. Surface Chl *a*

concentrations, measured by underway fluorescence, were uniformly low throughout our sampling region (mean=0.5 $\mu\text{g L}^{-1}$, values ranged from 0 to 1.8 $\mu\text{g L}^{-1}$). No obvious trends in underway surface Chl *a* fluorescence were observed along the river transect. There were no phytoplankton blooms present in the lower Sacramento River during our sampling.

The Fv/Fm in surface waters varied throughout the river transect (Figure 5-5), ranging from 0.13 to 0.58. Fv/Fm steadily increased upstream of the effluent outfall; the maximum value was at the far upstream end of the transect near Station A. Fv/Fm values averaged 0.45 ± 0.07 upstream from the effluent outfall pipe location. Fv/Fm downstream of the effluent outfall pipe in the lower Sacramento River averaged 0.27 ± 0.05 , significantly lower than Fv/Fm above the outfall ($p < 0.01$). However, the outfall did not appear to induce a stepwise change in Fv/Fm, as values steadily declined downstream from Station A and the confluence of the Sacramento and American Rivers. Fv/Fm declined in portions of the transect that received no exposure to effluent (e.g., in reaches upstream from the outfall pipe, but downstream from the confluence).

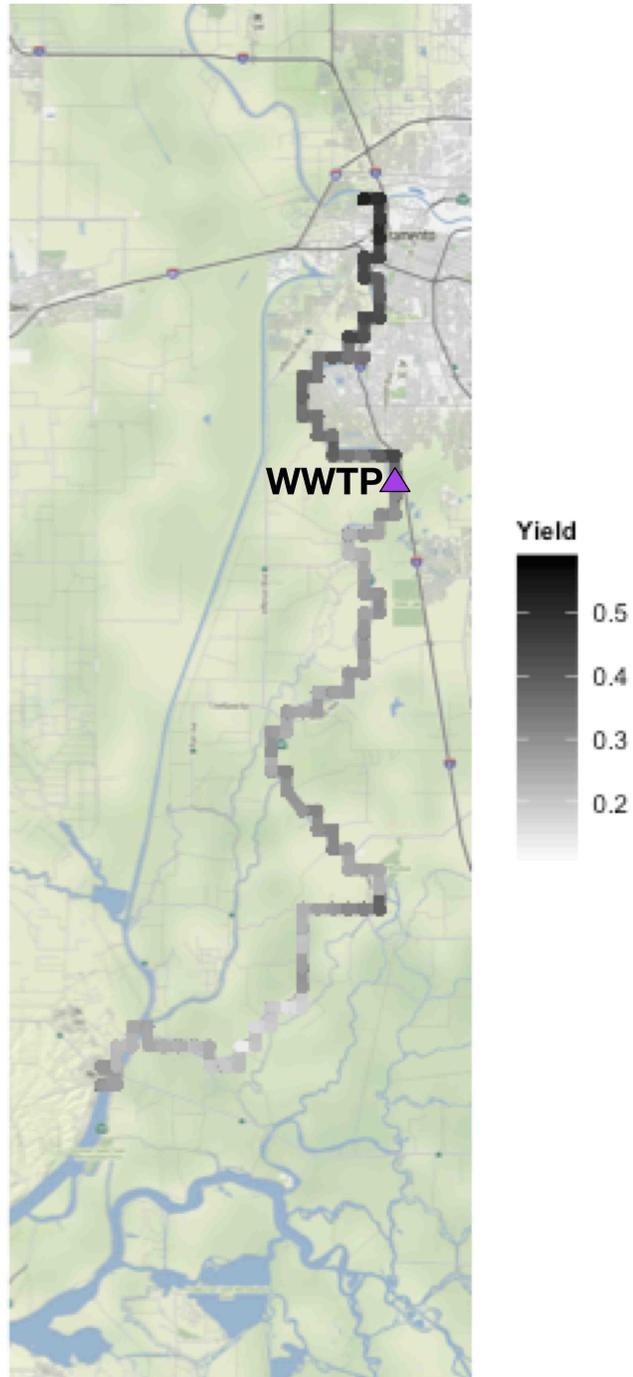


Figure 5-5 Variable fluorescence (F_v/F_m) yield is shown along the Sacramento River transect from 06 May 2015.

Surface DIN along transects sampled on 5-6 May 2015 showed a consistent pattern in the lower Sacramento River (Figure 5-6). NH_4^+ concentrations ranged from 0-3 μM upstream from the effluent outfall and from 40-65 μM in the river downstream of the outfall. Once lower Sacramento River water tidally mixed with waters from Suisun Bay and the San Joaquin River, just upstream from Rio Vista in the Cache Slough Complex, NH_4^+ concentrations dropped to <15 μM .

Surface NO_3^- concentrations upstream from the effluent outfall were 2-7 μM . Downstream from the outfall, NO_3^- concentrations steadily increased to approximately 30 μM (Figure 5-6). Once lower Sacramento River water tidally mixed with waters from Suisun Bay and the San Joaquin River, just upstream from Rio Vista in the Cache Slough Complex, NO_3^- concentrations increased to ~45 μM , due to the large contribution of high- NO_3^- San Joaquin River water.

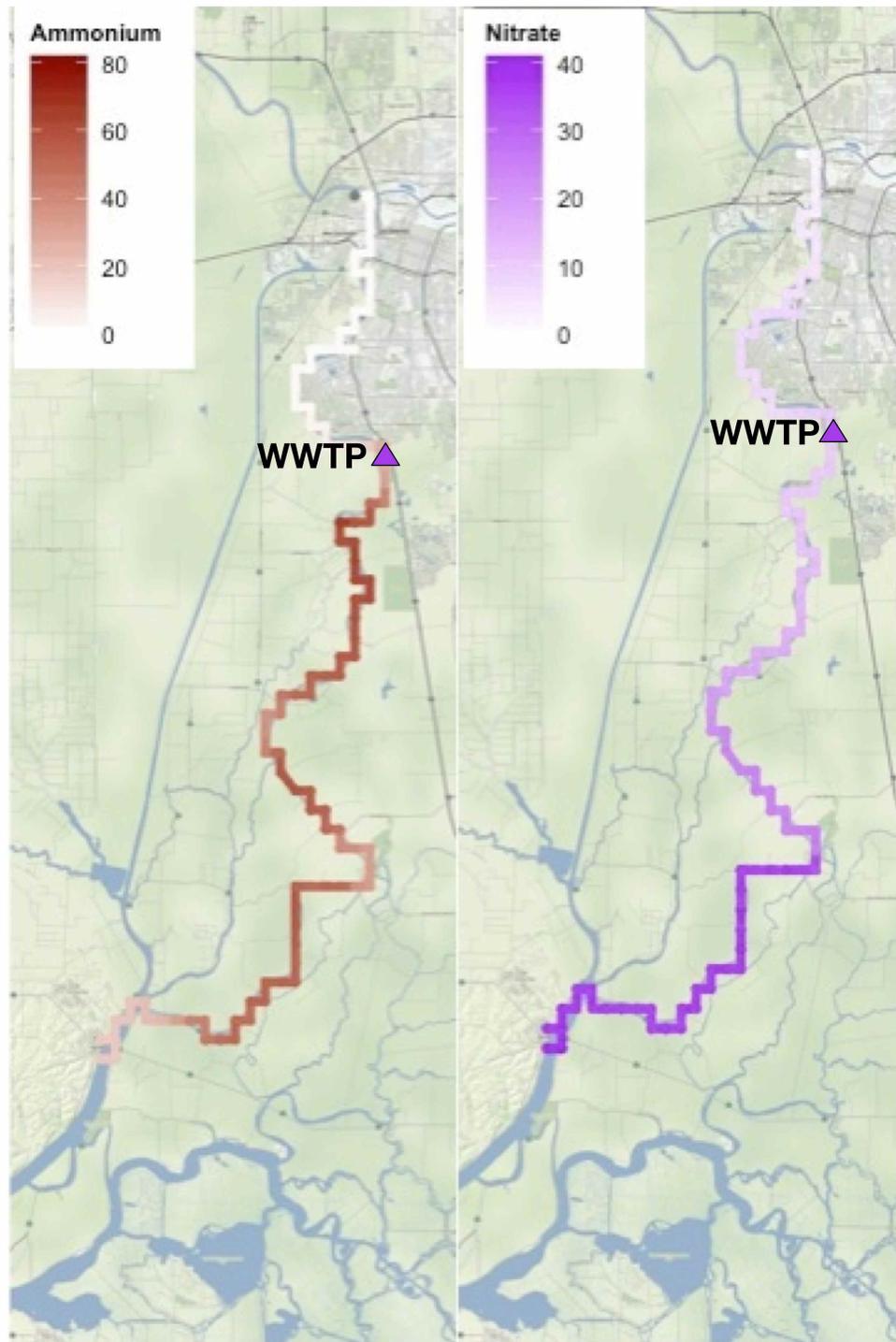


Figure 5-6. Surface DIN concentrations are shown along the Sacramento River transect from 06 May 2015 for NH_4^+ and NO_3^- .

Table 5-1. Initial nutrient conditions for all stations and the amounts added for +NH₄⁺, +NO₃⁻, and +Effluent treatments. Treatments were targeted to concentrations measured using underway instrumentation during sampling transect. Station C is downstream from the outfall. Stations and B were located upstream of the outfall.

	NH ₄ ⁺ (μM)			PO ₄ ³⁻ (μM)		NO ₃ ⁻ (μM)	
Station C	56.7			2.2		7.8	
	Initial	+NH ₄	+Effluent	Initial	+Effluent	Initial	+NO ₃
Station A	0.8	55.4	44.6	0.6	1.5	2.9	7.9
Station B	1.7	47.5	41.2	0.8	1.8	6.7	10.6

Targeted nutrient and light treatments

Table 5-1 shows a comparison of the initial concentrations of NH₄⁺, NO₃⁻, and PO₄³⁻ for all nutrient treatments and the corresponding in situ concentrations measured in surface waters. The NH₄⁺ concentrations in the +NH₄⁺ treatment closely matched the measured concentrations downstream of the outfall pipe, while in the +Effluent treatment NH₄⁺ concentrations averaged about 80% of that measured downstream from the effluent outfall.

At the start of the experiment, the LL incubator received 11% of the irradiance of the HL incubator. Over the duration of the experiment, we measured the relative fraction of light that reached 0.15 m depth within both HL and LL incubators incubator (the average depth of a cubitainer). This allows a relative comparison of shading (but does not provide a continuous absolute measurement of PAR). During the

night, luminous flux into both incubators was 0. Over the course of the three daylight periods of the experiment, the LL incubator at 0.15 m depth averaged 8.9% of the luminous flux of the HL incubator, close to the target 10% LL relative to HL.

Water temperature in the HL incubator ranged from 19.0°C to 22.4°C, which was similar to ambient river conditions. Water temperature in the LL incubator ranged from 19.1°C to 21.3°C, having warmed less due to lower incoming solar radiation

Chlorophyll a and organic carbon accumulation

Initial Chl *a* concentrations were low, reflecting low phytoplankton abundance at all sites. At the start of the experiment, Chl *a* concentrations at Station A ($1.3 \pm 0.2 \mu\text{g Chl } a \text{ L}^{-1}$) were nearly twice as high as at either Station B ($0.7 \pm 0.1 \mu\text{g Chl } a \text{ L}^{-1}$) or Station C ($0.5 \pm 0.1 \mu\text{g Chl } a \text{ L}^{-1}$).

After incubation for 48-hours, Chl *a* concentration increased dramatically in all controls and treatments, and at all locations (Figure 5-7). The increase in Chl *a* was significantly greater under HL conditions (average 552% increase) than LL conditions (average 194% increase) ($p < 0.05$). The largest magnitude Chl *a* accumulation was under HL conditions in the +Effluent and +NH₄⁺ addition treatments for both Stations A and B, and in the un-amended water from Station C, downstream of the outfall pipe. Under HL conditions, Chl *a* in the +Effluent treatment from Station A increased from just over $1 \mu\text{g Chl } a \text{ L}^{-1}$ at the start of the experiment to $7.5 \mu\text{g Chl } a \text{ L}^{-1}$, a 7-fold increase over 48 hours. Accumulation of Chl *a* L⁻¹ across the five sampling time points of the experiment is shown in Figure 5-8.

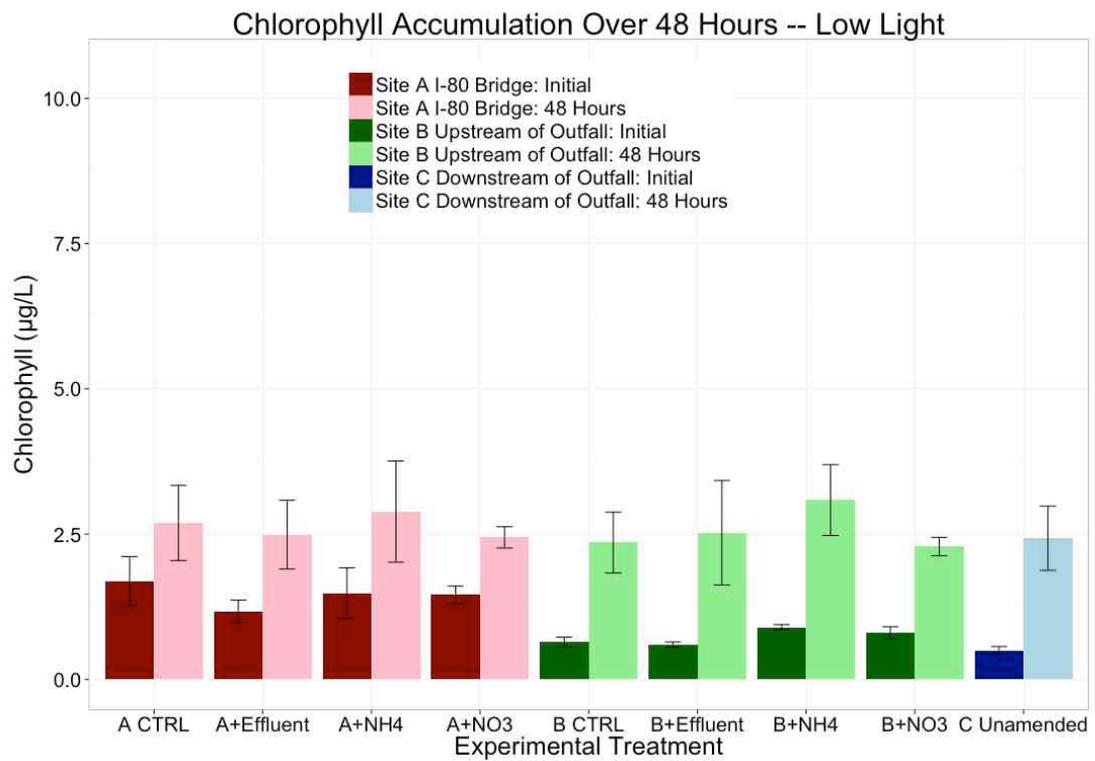
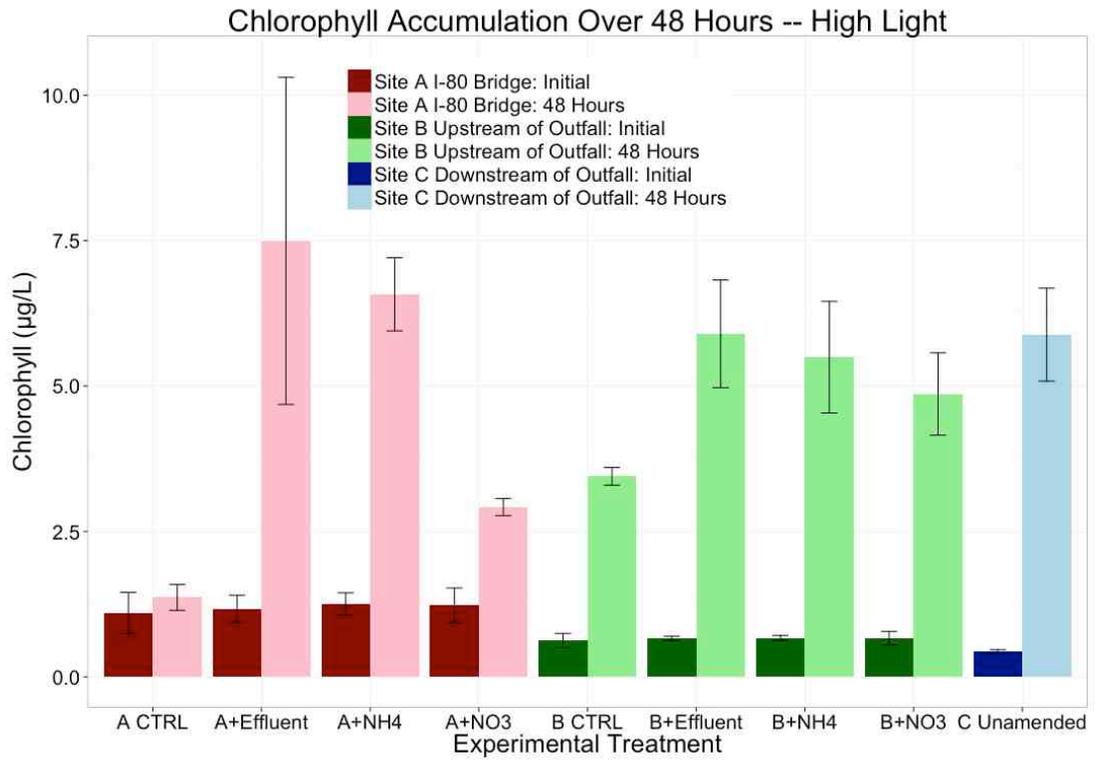


Figure 5-7. Chlorophyll *a* accumulation relative to initial conditions after 48-hours for all treatments is shown for HL (A) conditions and LL (B) conditions.

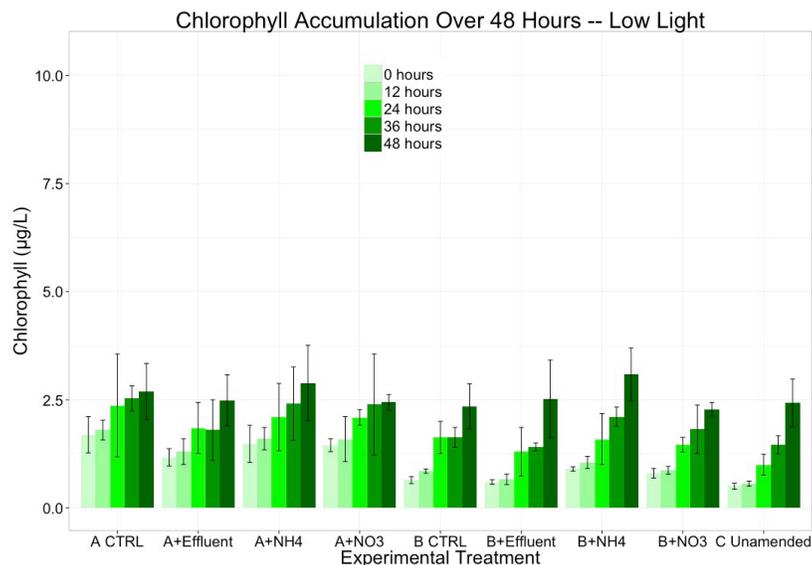


Figure 5-8. Time point-by-time-point Chl *a* accumulation across the experiment showing exponential growth for HL conditions (A) and LL conditions (B).

Specific growth rates (d^{-1}) calculated using increases in Chl *a* concentration over time were ranged from to $0.17 d^{-1}$ in the unamended water from Station A to $1.30 d^{-1}$ in the water from Station C, downstream from the wastewater effluent outfall (Table 5-2).

The pattern of Chl *a* accumulation in the experimental incubations was matched by increases in POC. POC accumulation and Chl *a* accumulation after 48 hours were significantly positively correlated ($p < 0.05$, $R^2 = 0.75$), and POC accumulation increased in all nutrient treatments under both HL and LL conditions. Under HL conditions, across all sampling locations and treatments, POC increases over 48 hours were significantly greater under HL conditions (mean = $1004 \pm 283 \mu g C L^{-1}$) than under LL conditions ($86 \pm 42 \mu g C L^{-1}$, $p < 0.01$, Fig 5-9). Just as for Chl *a* accumulation, POC increases under HL conditions were greatest in the +Effluent and +NH₄⁺ addition

treatments at both Stations A and B, and in the un-amended water from Station C, downstream of the effluent outfall.

Table 5-2. Growth rates and phytoplankton physiological parameters for all samples in (A) high light and (B) low light conditions.

A. High Light

Station + Treatment	Growth Rate μ (d ⁻¹)	Fv/Fm		Sigma (nm)	
		t=0	t=48	t=0	t=48
Station A Unamended	0.17±0.17	0.51	0.51	459.1	532.0
Station A + NH₄⁺	0.83±0.07	0.49	0.53	469.6	485.8
Station A + NO₃⁻	0.44±0.14	0.51	0.53	459.8	510.5
Station A + Effluent	0.91±0.11	0.49	0.50	473.5	497.3
Station B Unamended	0.86±0.11	0.50	0.54	467.4	502.0
Station B + NH₄⁺	1.05±0.08	0.52	0.50	451.5	496.0
Station B + NO₃⁻	1.00±0.10	0.50	0.54	466.7	506.3
Station B + Effluent	1.09±0.10	0.48	0.54	487.5	496.2
Station C Unamended	1.30±0.05	0.48	0.55	472.2	491.5

B. Low Light

Station + Treatment	Growth Rate μ (d ⁻¹)	Fv/Fm		Sigma (nm)	
		t=0	t=48	t=0	t=48
Station A Unamended	0.23±0.07	0.53	0.54	461.9	496.6
Station A + NH₄⁺	0.33±0.01	0.51	0.51	484.0	491.9
Station A + NO₃⁻	0.26±0.01	0.53	0.48	476.9	503.6
Station A + Effluent	0.37±0.06	0.51	0.50	455.4	496.4
Station B Unamended	0.65±0.15	0.53	0.44	474.8	531.3
Station B	0.61±0.07	0.53	0.45	479.8	529.1

+ NH₄⁺					
Station B + NO₃⁻	0.53±0.10	0.51	0.49	438.7	519.5
Station B + Effluent	0.70±0.16	0.50	0.54	479.6	509.2
Station C Unamended	0.78±0.05	0.49	0.55	489.2	510.3

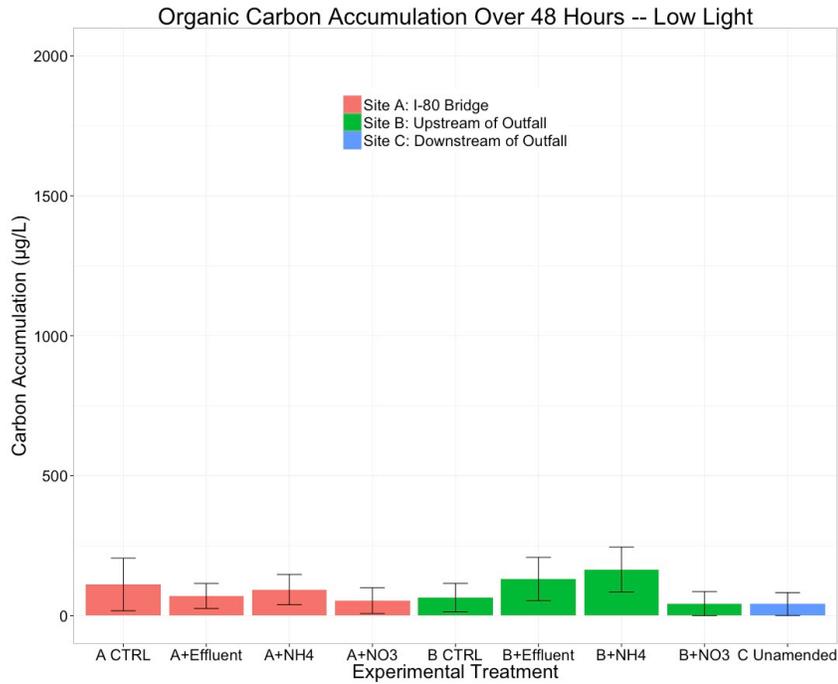
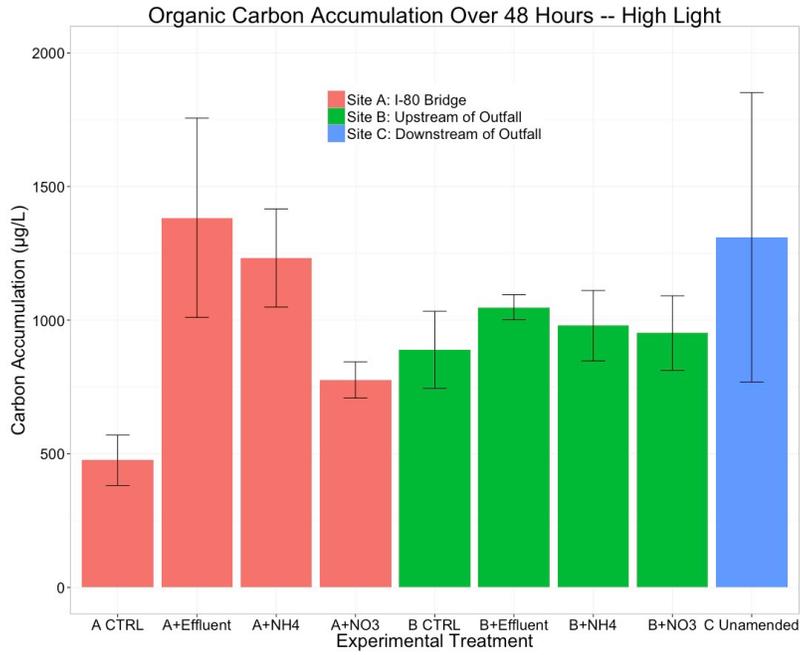


Figure 5-9. POC accumulation over 48 hours ($t=48 - \text{Initial}$) for HL (A) and LL (C) conditions.

Phytoplankton community composition

The initial phytoplankton communities in surface water at Stations A, B, and C were similar. Diatom species dominated cell abundances at all three locations (54%, 55%, 66% of all cells at Stations A, B, and C, respectively), principally *Thalassiosira* spp. Chlorophytes, principally *Chlorella* spp, were the next most abundant taxon at all stations (37%, 34%, 25%, of cell densities at stations A, B and C, respectively). Cryptophytes, principally *Rhodomonas* spp., (5%, 6%, 5% of cells at Stations A, B, and C, respectively), and cyanobacteria, principally *Anabaena* spp., (4%, 5%, 4% of cells at Stations A, B, and C, respectively) were also present in initial samples in similar abundances. Together, these four taxonomic groupings comprised over 99% of all cells in all samples. Because diatom species have larger cells, diatoms dominated the initial total biovolume of phytoplankton within the community (90% of cell biovolume at Station A, 96% at Station B, and 90% at Station C).

After 48 hours of growth, total phytoplankton cell densities significantly increased at all stations and under all treatments, under both HL and LL conditions (Figure 5-10). Across all sampling locations and treatments, total cell densities increased by an average of 20-fold, from 4.5×10^6 cells L⁻¹ to 8.3×10^7 cells L⁻¹ under HL conditions, and an average of 8-fold, from 4.5×10^6 cells L⁻¹ to 3.4×10^7 cells L⁻¹ under LL conditions, or slightly less than half as much growth as in HL (Table 3). Under HL conditions, cell densities showed the greatest increase in the +NH₄⁺ treatment with water from Station A, and in the un-amended treatment at Station C, downstream from the effluent outfall (Table 5-3).

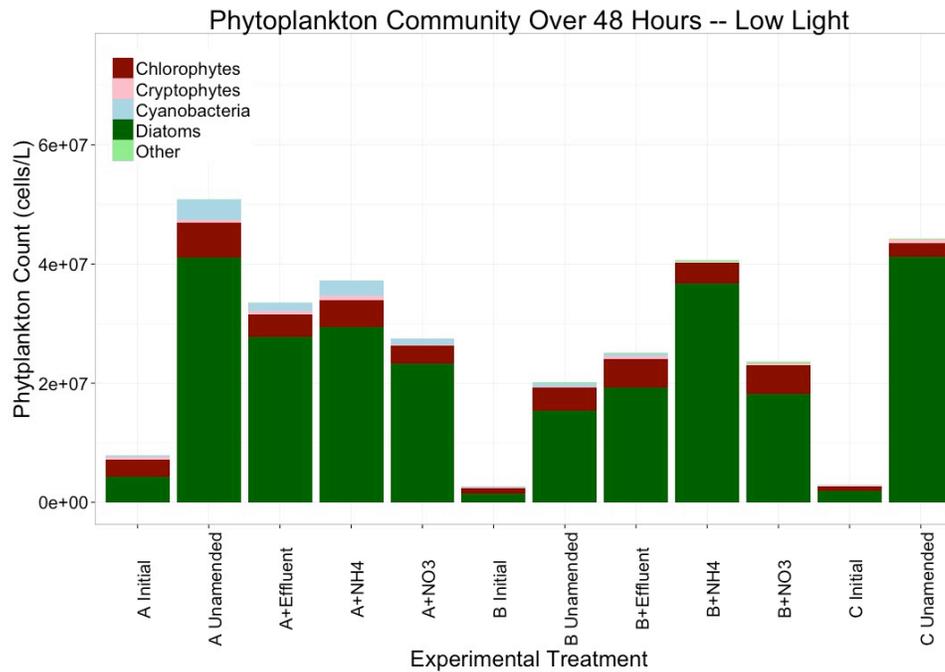
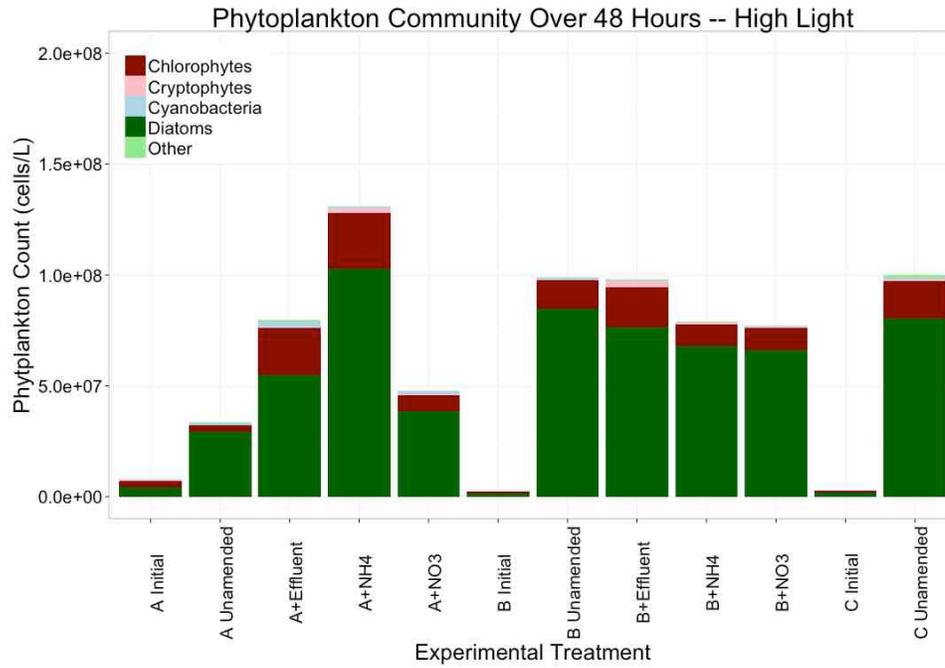


Figure 5-10. Phytoplankton cell abundances for all treatments under HL (A) and LL (B) conditions.

Diatom species grew rapidly under all treatment and light conditions, increasing by an average of 11-fold. The fraction of total cells that were diatoms increased across all controls, treatments and light conditions after 48 hours (Table 4). While chlorophyte cell counts increased substantially across all treatments, the relative cell densities of chlorophytes within the phytoplankton community decreased over 48 hours in all treatments and light conditions, due to the more substantial increases of diatom cell densities (Table 5-4).

Table 5-3. Cell Densities and Chlorophyll per Cell under (A) High Light and (B) Low Light.

Stations and Treatment	Cell Density (10^6 cells L^{-1})		Chlorophyll Per Cell (μg Chl a $cell^{-1}$)	
	High Light T=48	Low Light T=48	High Light T=48	Low Light T=48
Station A Initial	7.89		0.50	
Station B Initial	2.69		0.77	
Station C Initial	2.89		0.49	
Station A Unamended	33.5	50.9	0.12	0.16
Station A + NH_4^+	131	37.3	0.15	0.23
Station A + NO_3^-	47.7	27.5	0.18	0.27
Station A + Effluent	79.7	33.5	0.28	0.22
Station B Unamended	98.8	20.2	0.10	0.35
Station B + NH_4^+	78.8	40.6	0.21	0.23
Station B + NO_3^-	77.1	23.6	0.19	0.29
Station B + Effluent	98.0	25.1	0.18	0.30
Station C Unamended	100	44.3	0.18	0.16

Cyanobacteria cell counts increased by an average of 3-fold, with large increases in all treatments under HL conditions. Exceptions were HL conditions for the samples

from Station C (initial and final) and the LL samples from the $+NO_3^-$ and $+NH_4^+$ treatments from Station B, where no cyanobacterial cells were found after 48 hours. Interestingly, across all treatments there was no large increase in the cell density of cryptophytes, and cryptophyte cell counts actually decreased over 48 hours in the Unamended and $+NO_3^-$ treatments from Station A under HL conditions. Cryptophyte cell counts were uniformly low.

Overall, the three most abundant genera in all of our samples were *Thalassiosira* (a diatom), *Chlorella* (a chlorophyte), and *Anabaena* (a cyanobacterium). Together, these three genera comprised 55-80% of all phytoplankton cells in all of our treatment conditions and samples. Of these three genera, *Thalassiosira* and *Anabaena* showed the most dramatic cell division and growth rates, consistent with bloom initiation, under both high- and low- NH_4^+ treatment conditions (Figure 5-11).

Table 5-4. Relative Cell Densities of Phytoplankton Taxa for (A) High Light conditions and (B) Low Light conditions.

A. High Light

	Diatoms	Chlorophytes	Cryptophytes	Cyanobacteria	Other
Station A Initial	54.1%	37.1%	4.8%	3.9%	0.1%
Station A Unamended 48 hours	87.4%	8.8%	0.2%	3.0%	0.6%
Station A + NH_4^+ 48 hours	78.6%	19.1%	1.5%	0.7%	0.1%
Station A + NO_3^- 48 hours	81.0%	15.1%	0.6%	3.3%	0.0%
Station A + Effluent 48 hours	68.8%	26.5%	0.5%	3.3%	1.0%
Station B Initial	54.8%	34.3%	5.5%	5.3%	0.1%
Station B Unamended	86.0%	12.9%	0.5%	0.5%	0.1%

48 hours					
Station B + NH₄⁺ 48 hours	86.6%	11.8%	1.2%	0.1%	0.2%
Station B + NO₃⁻ 48 hours	85.7%	13.0%	0.4%	0.6%	0.3%
Station B + Effluent 48 hours	78.2%	18.4%	2.7%	0.5%	0.3%
Station C Initial	66.0%	24.9%	5.0%	3.7%	0.4%
Station C Unamended 48 hours	80.1%	17.0%	0.8%	0.6%	1.5%

B. Low Light

	Diatoms	Chlorophytes	Cryptophytes	Cyanobacteria	Other
Station A Initial	54.1%	37.1%	4.8%	3.9%	0.1%
Station A Unamended 48 hours	81.0%	11.2%	0.9%	6.8%	0.1%
Station A + NH₄⁺ 48 hours	79.0%	12.2%	2.0%	6.8%	0.1%
Station A + NO₃⁻ 48 hours	84.5%	11.4%	0.4%	3.7%	0.0%
Station A + Effluent 48 hours	83.0%	11.0%	1.8%	4.0%	0.2%
Station B Initial	54.8%	34.3%	5.5%	5.3%	0.1%
Station B Unamended 48 hours	76.2%	19.1%	1.0%	3.2%	0.7%
Station B + NH₄⁺ 48 hours	90.5%	8.4%	0.4%	0.0%	0.6%
Station B + NO₃⁻ 48 hours	77.3%	20.5%	1.2%	0.0%	1.0%
Station B + Effluent 48 hours	76.8%	18.8%	2.1%	1.7%	0.7%
Station C Initial	66.0%	24.9%	5.0%	3.7%	0.4%
Station C Unamended 48 hours	93.0%	5.3%	1.3%	0.0%	0.4%

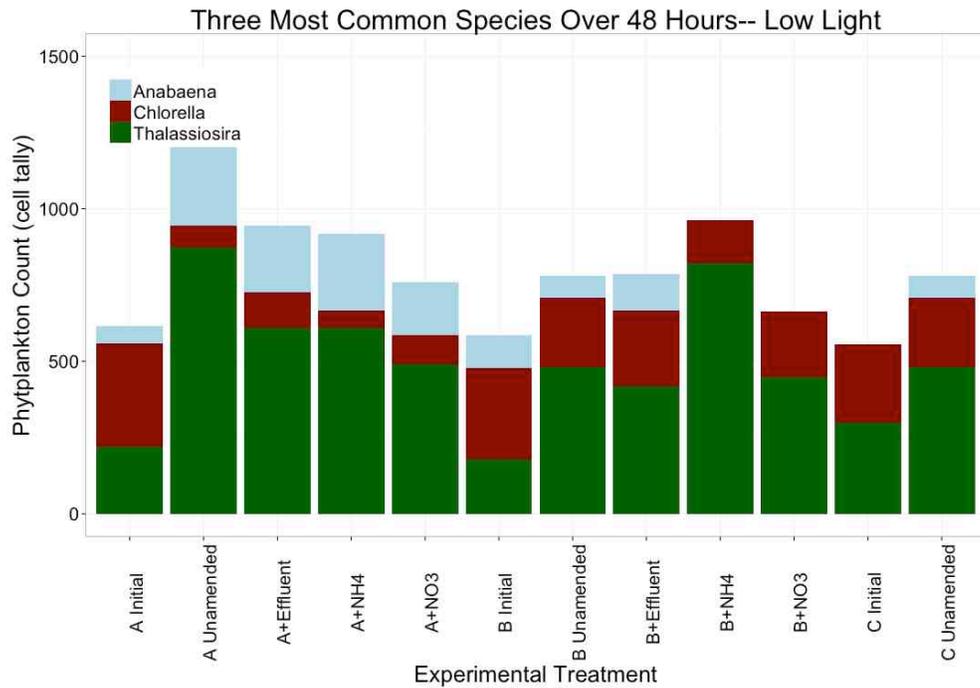
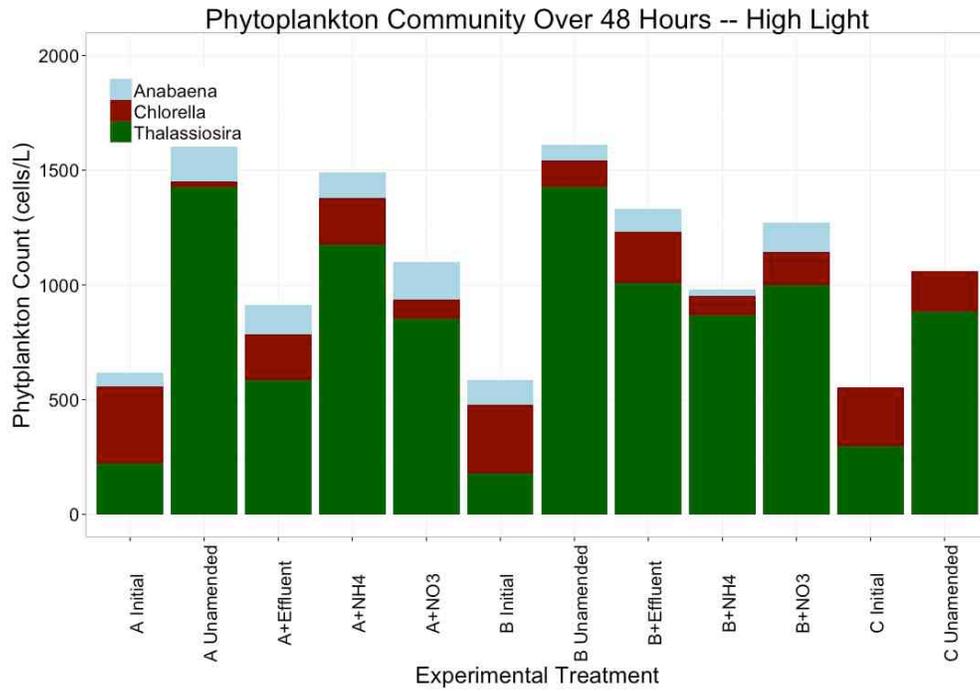


Figure 5-11 Cell densities for three most abundant phytoplankton species under HL (A) and LL (B) conditions.

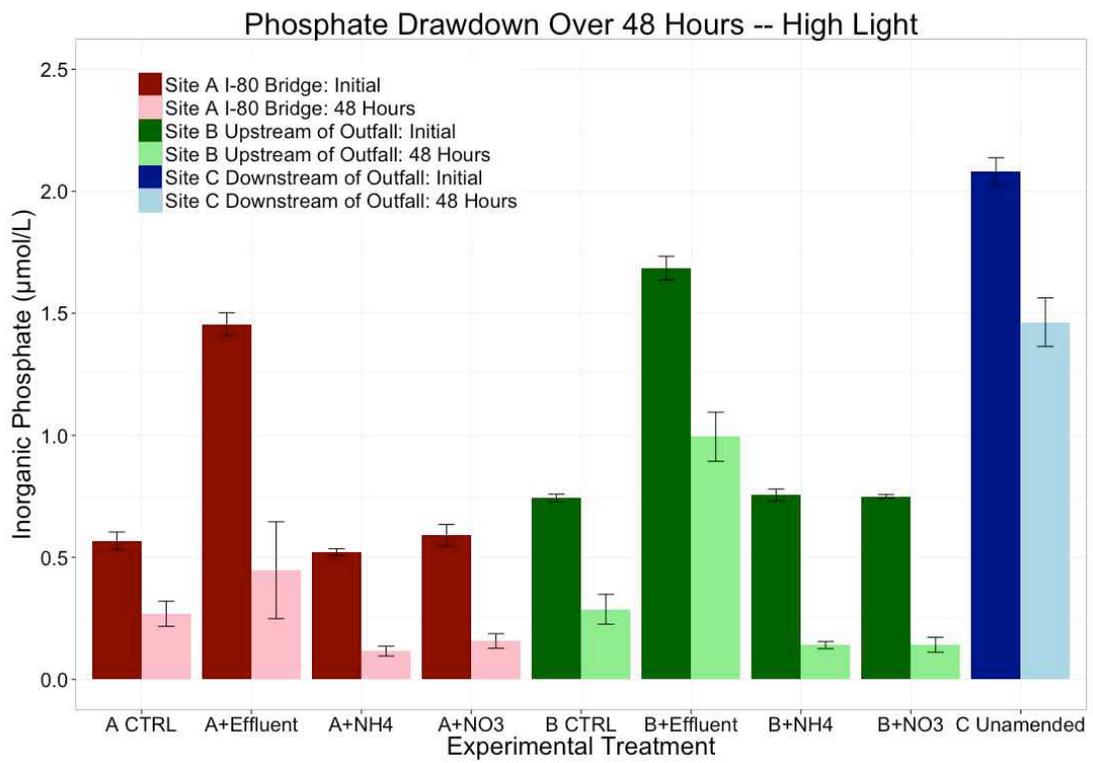
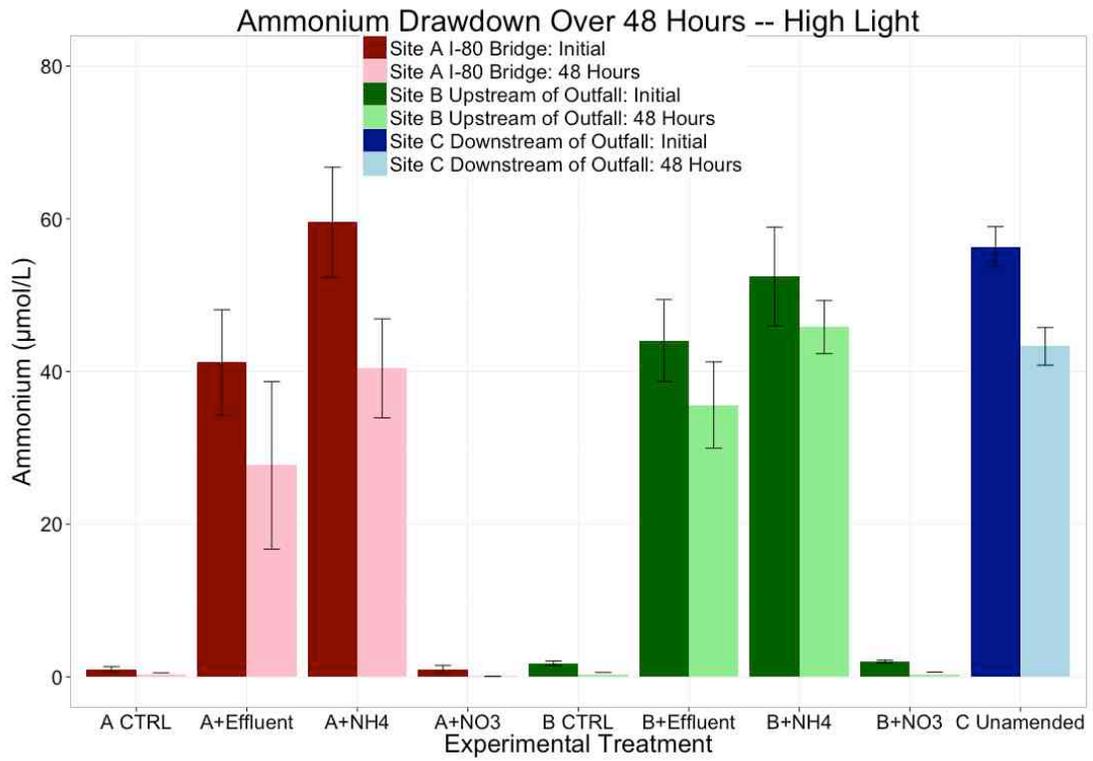
Carbon:Chlorophyll and Chlorophyll per cell

Light treatments influenced the Carbon:Chl *a* (C:Chl *a*) ratio in our incubated samples. Because initial detrital and non-phytoplankton organic C concentrations were high (initial C:Chl *a* = 706±276), C:Chl *a* ratios as Δ POC: Δ Chl *a* were measured at t=24 hours and at t=48 hours. Δ POC: Δ Chl *a* at t=24 hours was significantly greater (176±95 μ g C: μ g Chl *a*) under HL conditions across all stations and treatments compared to LL conditions (65±42 μ g C: μ g Chl *a*, $p < 0.05$). Δ POC: Δ Chl *a* between t=0 and t=48 hours incubation was also significantly greater (262±88 μ g C: μ g Chl *a*) under HL than LL conditions (58±26 μ g C: μ g Chl *a*, $p < 0.05$). No significant effects of sampling station or nutrient treatment on C:Chl *a* ratios were observed. Initial Chl *a* per cell at the start of the experiment was greater at all three sampling stations than Chl *a* per cell after 48 hours. Across all samples, Chl *a* per cell at the start of the experiment was 0.60 pg Chl *a* cell⁻¹, decreasing to 0.21 pg Chl *a* cell⁻¹ with a range of 0.12 to 0.35 pg Chl *a* cell⁻¹ at the end of the experiment. Chl *a* per cell was, on average, greater under LL conditions after 48 hours than under HL conditions, although this was not true for all treatments or at all stations (see Table 5-3).

Nutrient drawdown

The Chl *a* accumulation and increases in POC observed over 48-hours were also matched by drawdown of inorganic nutrients (DIN, PO₄³⁻ and dSi) (Figures 12A, 12B, 12C). For all nutrients, drawdown over 48 hours was significantly greater under HL conditions relative to LL conditions ($p < 0.05$). Initial PO₄³⁻ concentrations were

greatest in the +Effluent and un-amended Station C (downstream of outfall) samples. Available PO_4^{3-} was not completely consumed in any treatment. Notably, for PO_4^- and dSi drawdown there were minimal differences between treatments. For the five HL treatment/station combinations with NH_4^+ replete concentrations (initial conditions $>40 \mu\text{M NH}_4^+$), which included Station A+ NH_4^+ , Station A+Effluent, Station B+ NH_4^+ , Station B+Effluent, and Station C Un-amended, the amount of NH_4^+ removed over 48 hours ranged from 12-33% of the available NH_4^+ pool. The only location where DIN appeared to have become limiting was at Station A in the un-amended treatment in which Chl *a* accumulation peaked at 36 hours and then declined to 48 hours, and where both NH_4^+ and NO_3^- concentrations dropped to below detection at the end of the experiment. All other sampling treatments/locations retained some DIN at the end of the experiment.



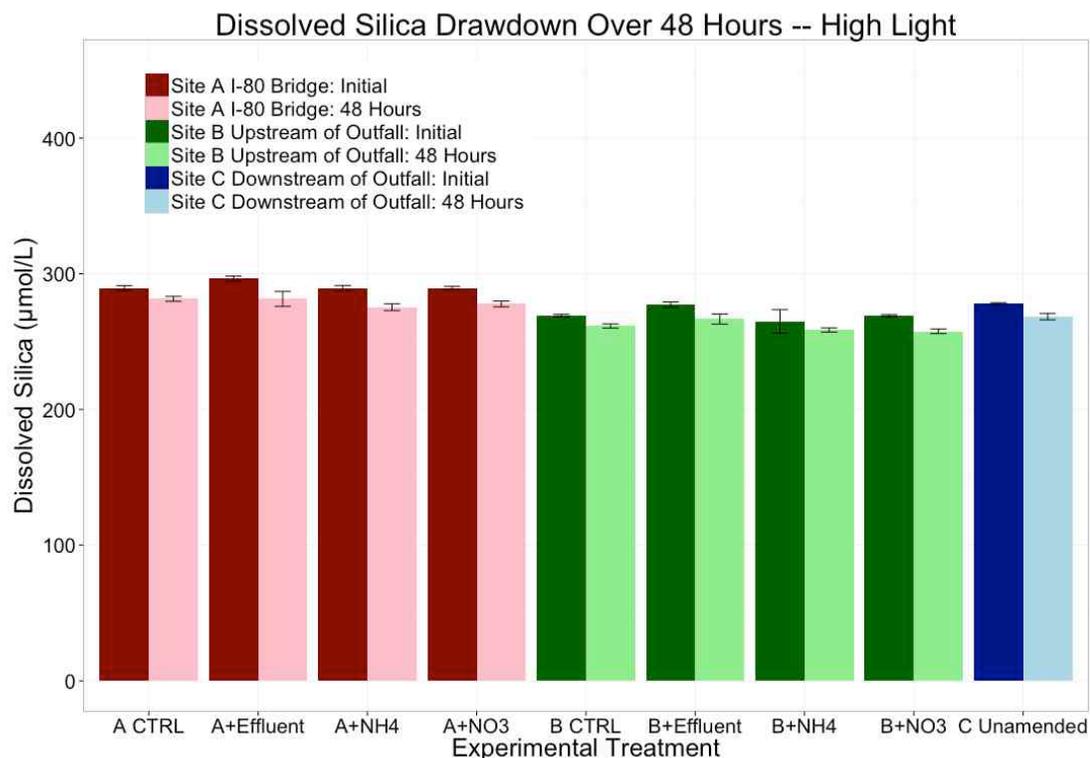


Figure 12. Nutrient drawdown over 48 hours under HL conditions for NH_4^+ (A), PO_4^{3-} (B), and dSi (C)

Phytoplankton physiology

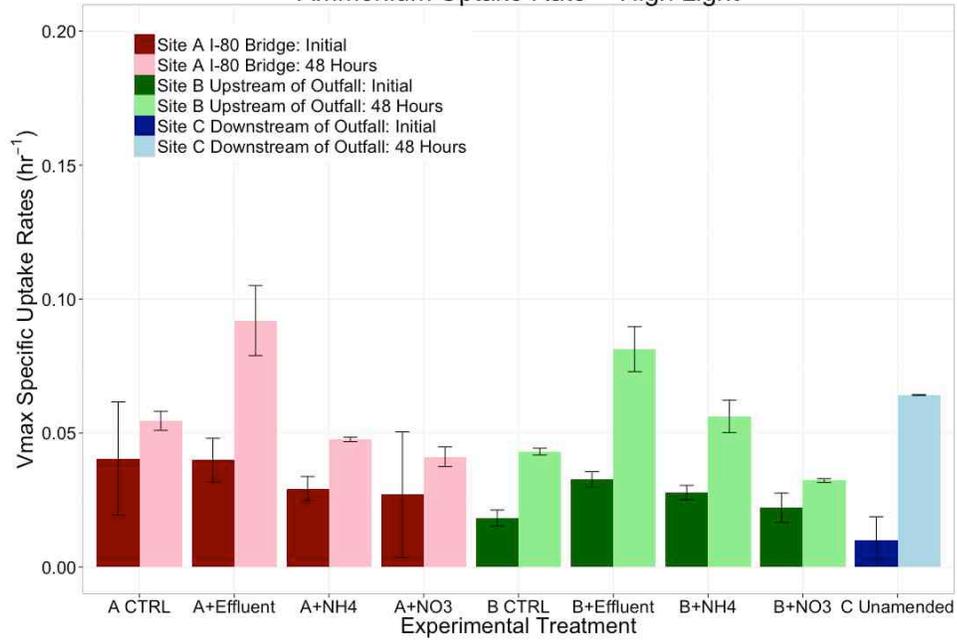
Fv/Fm did not vary significantly during the incubation experiment, either across nutrient or light treatments, or across time. Values in all samples were uniformly high, between 0.45 and 0.55 (Table 5=2). Across all treatments, functional absorption cross sections (σ_{PSII}) increased significantly ($p < 0.05$) over the 48 hours of the experiment (Table 5=2).

^{15}N uptake rates

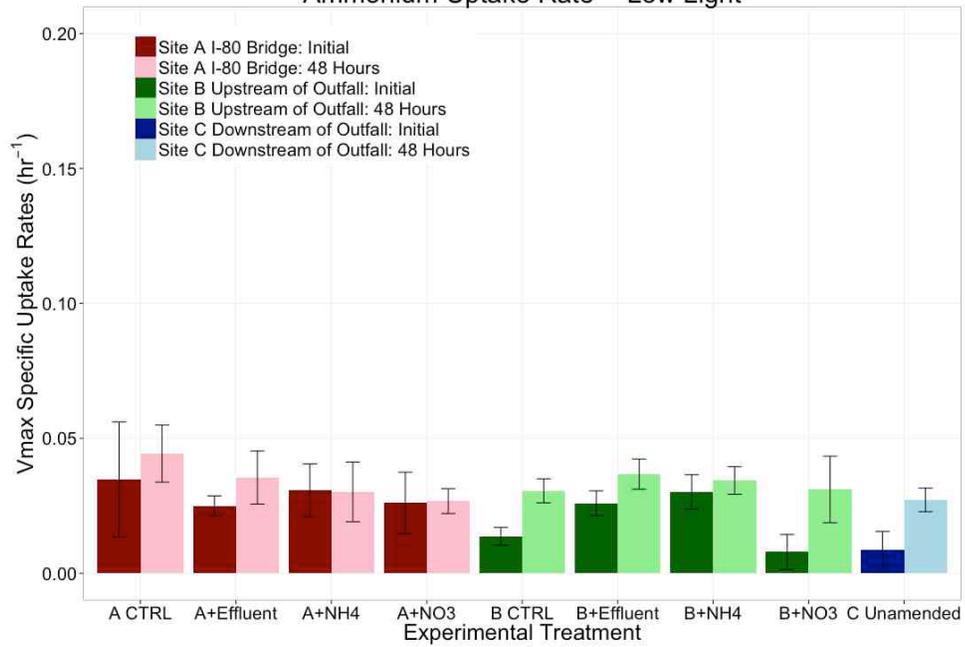
Maximal specific uptake rates (V_{max}) for NH_4^+ increased over the 48 hour incubations in all treatments. NH_4^+ uptake rates were significantly greater in HL than in LL conditions (Fig. 13A,B). In contrast, NO_3^- uptake rates were completely

suppressed in the presence of NH_4^+ when added alone, in the +Effluent treatments, and in the un-amended water from downstream of the outfall site (Fig. 13C,D). NO_3^- uptake rates were greatest in the + NO_3^- treatments for Stations A and B (to which no NH_4^+ was added and in which starting concentrations of NH_4^+ were $<5 \mu\text{M}$). NO_3^- uptake rates also were low, but greater than zero, in the un-amended Station A and B samples, which had low (but non-zero) concentrations of both NH_4^+ and NO_3^- at the start of the experiment. Overall, there was no significant difference in the V_{max} of NH_4^+ and NO_3^- ($p>0.05$), although the highest DIN uptake rates we observed were for NO_3^- uptake in the + NO_3^- treatments. Absolute uptake rates (ρ , $\mu\text{mol DIN L}^{-1} \text{hr}^{-1}$) for all treatment and locations at $t=48$ hours were highest in the +Effluent ($2.21 \mu\text{mol DIN L}^{-1} \text{hr}^{-1}$) and + NH_4^+ treatments ($1.93 \mu\text{mol DIN L}^{-1} \text{hr}^{-1}$) under HL conditions (Table 5-5).

Ammonium Uptake Rate -- High Light



Ammonium Uptake Rate -- Low Light



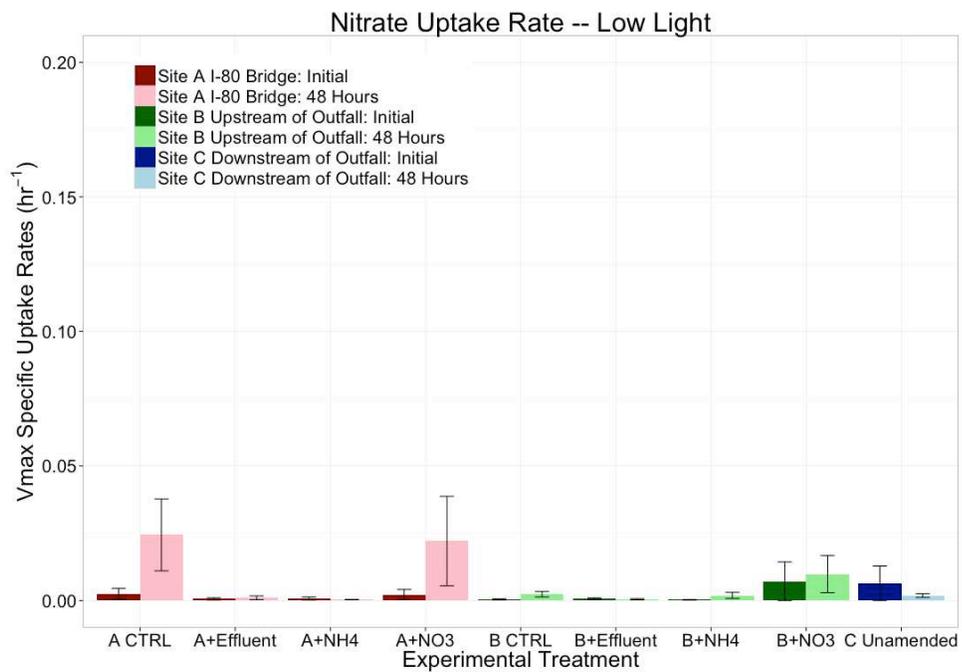
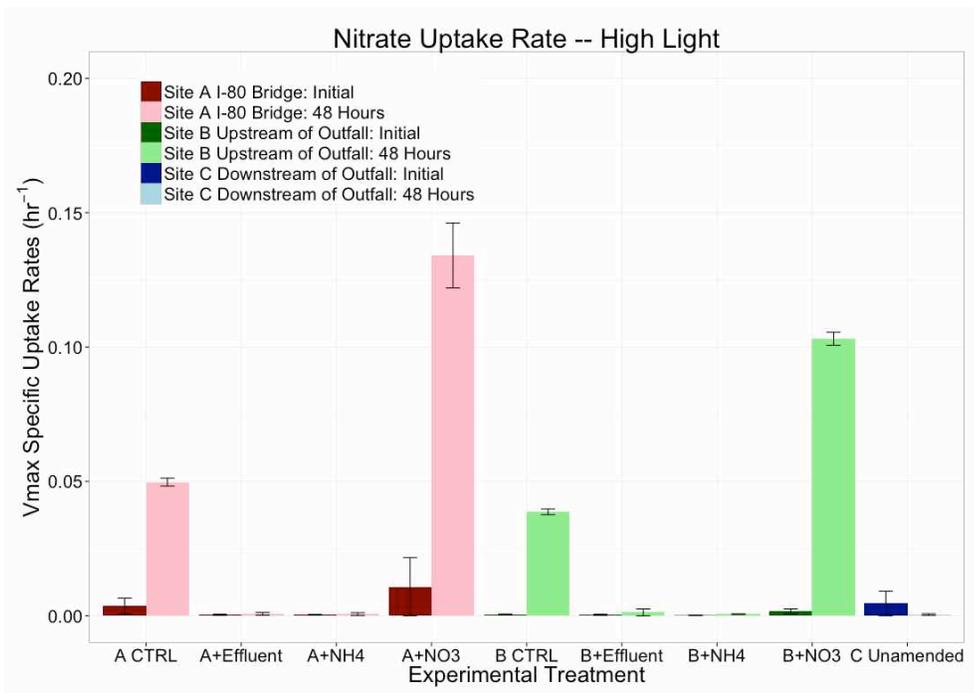


Figure 5-13. Specific uptake rates for NH_4^+ are shown under HL conditions (A) and LL conditions (B) for all treatments for initial and $t=48$ hours. Specific uptake rates for NO_3^- are shown under HL (C) and LL (D) conditions for all treatments for initial and $t=48$ hours.

Table 5-5. DIN Uptake Rates at 48 Hours

Station + Treatment	High Light ρ ($\mu\text{mol DIN L}^{-1} \text{h}^{-1}$)		Low Light ρ ($\mu\text{mol NO}_3^- \text{L}^{-1} \text{h}^{-1}$)	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-
Station A Unamended	0.40	0.37	0.30	0.17
Station A + NH_4^+	0.86	0.01	0.20	0.00
Station A + NO_3^-	0.43	1.39	0.18	0.15
Station A + Effluent	1.93	0.01	0.23	0.01
Station B Unamended	0.49	0.45	0.17	0.01
Station B + NH_4^+	0.81	0.01	0.22	0.01
Station B + NO_3^-	0.39	1.26	0.19	0.06
Station B + Effluent	1.23	0.02	0.24	0.00
Station C Unamended	2.21	0.01	0.15	0.01

DISCUSSION

We present clear evidence of phytoplankton growth on wastewater effluent from the SRWTP. Contrary to the findings of several previous studies in the region (Dugdale et al. 2007; Parker et al. 2012), phytoplankton growth, carbon fixation and accumulation, Chl *a* production, cell division, and nutrient drawdown, were all measured at high NH_4^+ concentrations (above 40 μM). Under both our HL and LL treatments, phytoplankton grew rapidly when cells were supplied with nutrients from effluent, with “clean” NH_4^+ , and with ambient nutrient concentrations of Sacramento River water downstream from the sanitation district effluent outfall.

Consistent with numerous laboratory and in situ studies from other estuaries, as well as previous work in the lower Sacramento River, high concentrations of NH_4^+ , whether present in the river or experimentally added from clean NH_4^+ or effluent,

suppressed NO_3^- uptake rates, which were near 0 in all samples with high NH_4^+ conditions. Yet the phytoplankton communities exposed to high NH_4^+ conditions grew well on NH_4^+ over the 48 hours of our experiment. NH_4^+ uptake rates were high and similar to NO_3^- uptake rates in samples without high NH_4^+ concentrations, and the amount of phytoplankton biomass produced from NH_4^+ uptake was similar to the amount of phytoplankton biomass produced by NO_3^- uptake. Measurements of Fv/Fm throughout the experiment showed that phytoplankton communities under these high NH_4^+ experimental conditions had well functioning photosystems. Overall, rates of DIN uptake were similar to those previously observed in this system (Parker et al. 2012). While our experiment only lasted for two days, the rapid accumulation of Chl *a* and phytoplankton biomass during this time (cells were doubling at least once a day in many of our treatments under HL conditions) suggests that bloom conditions had been initiated within our nutrient replete incubations, including un-amended water from the lower Sacramento River (Figure 5-8). In short, the only treatment required to initiate a bloom in the high- NH_4^+ river water from downstream of the effluent outfall was removing cells from the turbid and turbulent environment of the lower Sacramento River.

Our experimental results strongly indicate that the paradoxically low in situ phytoplankton growth in the lower Sacramento River, despite relatively high ambient concentrations of DIN from anthropogenic inputs, occurs due to light limitation (at least in the absence of benthic grazers). The largest effect on phytoplankton growth, carbon fixation, Chl *a* accumulation, nutrient drawdown, DIN uptake, and cell division in our experiment was caused by a change in irradiance, not by changes in total

nutrient concentrations or DIN speciation. When held at light levels equivalent to those at 3 to 4 m depth in a well-mixed 10 m water column, phytoplankton growth was a small fraction of that when cells were held at light levels equivalent to 0.6-0.8 m depth.

Evidence of strong control by light is also provided by C:Chl *a* ratios and Chl *a* per cell. C:Chl *a* was much greater under HL than under LL conditions and Chl *a* per cell increased under LL conditions, relative to the HL environment, consistent with a light acclimation response (Cloern et al. 1995). Notably, both C:Chl *a* ratios and Chl *a* per cell indicated that our LL treatment represented an increase in light availability relative to the ambient conditions in the river. In all experimental conditions and light levels, Chl *a* per cell decreased and C:Chl *a* increased. This strongly suggests that the average light environment experienced by cells in both our LL conditions was still greater than the average light environment that cells would have experienced in situ. For example, Cloern et al. (1985) found that in Suisun Bay, the euphotic depth was only 10% of the total depth of the well-mixed water column, leading to phytoplankton losses exceeding growth.

Our experimental data also strongly suggest that the input of anthropogenic NH_4^+ into the river system from the effluent outfall pipe supports growth of most of the species within the phytoplankton community present in situ. In particular, the in situ ambient phytoplankton community in the lower Sacramento River at all of our sampling locations was dominated by diatom species, in terms of both cell density and biovolume, at the start of our experiment and this remained true after 48-hours under all light and treatment conditions.

Diatom species, in particular those of the genus *Thalassiosira*, grew readily on NH_4^+ , as evidenced by increases in cell density in experimental conditions where NO_3^- uptake rates were near-zero. Thus, under sufficient light, it is clear that diatoms grow well on the NH_4^+ that is present from wastewater loading in the SFBD. Furthermore, our analysis shows that diatom cell densities increased at greater rates than chlorophytes, in contrast to previous findings based on pigment analyses reported by Glibert et al. (2014b) and previous observations of upper Sacramento River blooms being dominated by chlorophytes (Glibert et al. 2014a).

Similarly to what has been previously reported, our findings show that cyanobacterial cell abundances increased under high-DIN conditions (Glibert et al. 2014b). However, the greater growth of cyanobacteria (and diatoms) was not unique to the addition of NH_4^+ or Effluent, relative to the addition of NO_3^- or controls, nor did such growth represent an ecologically meaningful shift in the phytoplankton community away from diatoms, though we do not know what longer incubation times might have revealed. Our microscopy data clearly show a phytoplankton community dominated in number and volume by diatom species under all light and nutrient growth conditions.

Taken as a whole, our experimental results present strong evidence that light availability exerts a major control on phytoplankton growth and bloom formation in the lower Sacramento River and that high concentrations of NH_4^+ from wastewater are not likely to be implicated as a cause of pelagic organism decline (POD) in the region. When water from the river downstream of the wastewater effluent outfall was exposed to light levels greater than those experienced in situ but with no other amendments,

phytoplankton community growth, driven primarily by *Thalassiosira*, was immediate, sustained, and significant. High NH_4^+ concentrations did not prevent a phytoplankton bloom from developing within our experiment and the form of inorganic N was not a major driver of phytoplankton community composition.

Grazing

Previous studies demonstrated that grazing by the invasive clam species *Potamocorbula amurensis*, introduced to Suisun Bay in the 1980s, played a significant role in inhibiting bloom formation in the SFBD (Thompson 2005). However, at X2 (a commonly used metric defined as the distance measured up the estuary from the Golden Gate where bottom water isohaline has a salinity of 2 in northern San Francisco Bay) there is a sharp decrease in the abundance of *P. amurensis* and this clam is not present in the freshwater reaches of the Delta. Instead, the Delta is dominated by the invasive clam *Corbicula fluminea*, which negatively impacts phytoplankton biomass and productivity in areas where it is abundant (Foe and Knight 1985, Lopez et al. 2006). Because our experimental design relied on incubations in cubitainers, which were pre-filtered through a 300 μm screen, we excluded the presence of both benthic grazers and macrozooplankton from our experimental bottles. Thus, we are unable to determine if grazing pressure is sufficient to inhibit bloom formation in the lower Sacramento River, even if light limitation were alleviated (Lucas et al. 2002). At the very least, some of the observed increase in production in the controls from the sites upstream the outfall pipe is likely due to a removal of grazing pressure. Further work should emphasize the role of grazing pressure in

controlling phytoplankton biomass and production in this region. Nonetheless, our results strongly show that light controls phytoplankton growth and production in situ in the Lower Sacramento River. If grazing exerts a further pressure on phytoplankton abundance, it would only serve to compound the influence of light observed here.

Drought

Our experiments and surface transects were performed during a record-setting drought in the state of California. The water temperatures we observed in early May in the lower Sacramento River are similar to those normally found in mid-July, and are near-record setting for that time of year (USGS, personal communication). Freshwater flow from both the Sacramento and San Joaquin Rivers in May 2015 was unusually low, and salinity values in the lower river near Rio Vista were among the highest ever recorded in USGS water quality datasets (the only year that salinity was greater was the 1976-77 drought). Indeed, daily X2 was generally upstream of 90 km from Golden Gate during spring 2015 (Monismith, submitted), a value that is ca. 10 km greater than long-term averages (Kimmerer 2004). Previous work in the region suggested that bloom formation is more likely to occur under low-flow conditions during droughts (Glibert et al. 2014a). Our data demonstrate that phytoplankton present under drought conditions where no bloom is found are capable of initiating a bloom, if given sufficient light. In a report on Suisun Bay, Kudela (2014) calculated PAR attenuation ($K_{PAR} \text{ m}^{-1}$) in the Lower Sacramento River and Suisun Bay, based on quarterly CTD and optics profiles in the region. Combining the depth of the water column, K_{PAR} , and estimations of phytoplankton E_k (the irradiance at which

phytoplankton cells transition from light to biochemical limitation of growth), Kudela (2014) found that nearly the entire region is significantly light limited. In particular, in well-mixed water columns such as those observed in our study, 80-90% of the water column is light-inhibited.

Nitrification and water quality management

In 2014, a new National Pollution Discharge Elimination System (NPDES) discharge permit was approved for the Sacramento Regional Sanitation District, which requires conversion of the wastewater treatment plant to a tertiary treatment system that will include both nitrification and denitrification steps (California Regional Water Board, Central Valley Region, 2014). These changes mean that the effluent outfall will no longer discharge N waste primarily as NH_4^+ , and that total dissolved N concentrations in the discharge will decrease by 74%. Data from our underway transect, showing gradual increase in surface NO_3^- concentrations downstream from the outfall pipe, suggest that the lower Sacramento River is a region of high rates of nitrification. This confirms findings from previous studies that have found high rates of nitrification in the lower Sacramento River, downstream of the effluent outfall (Parker et al. 2012, Glibert et al. 2014b, Damashek et al. 2015, 2016). Prior to discharge and dilution through tidal mixing in Suisun Bay, much of the anthropogenic NH_4^+ appears to be converted to NO_3^- in the water column. Thus, the ammonia-oxidizing microbial communities present in the lower Sacramento River may already be providing “nitrification services” over the stretch of the lower river, even before tertiary treatment comes online. Given the rapid growth of diatoms we observed on

anthropogenic NH_4^+ when light limitation was alleviated, our results highlight that we should not assume that future reductions in NH_4^+ loading from the tertiary treatment system would lead to an increase in the productivity of phytoplankton in the SFBD without any concomitant changes in the light environment. In fact, if the turbidity of the SFBD continues to decline, there may be cause for concern that reductions in turbidity could lead to eutrophication and hypoxia in the SFBD. Given anticipated agricultural N loads from the San Joaquin River, changes in the form and magnitude of WWTP loading under new permits, our increasing understanding of microbial N processing, and potential changes in freshwater flows under climate change and water usage scenarios, modeling the future nutrient environment of SFBD is a key direction for future research.

CONCLUSIONS

Our experimental results demonstrate that the phytoplankton community in the SFBD can grow well on either NH_4^+ or NO_3^- in this impacted estuarine system with high DIN concentrations from anthropogenic sources. These insights are critical to modeling efforts that seek to understand how SFBD will respond to climate change, changes in freshwater delivery, on-going reductions in sediment load, and to changes in the form of DIN delivered from wastewater. We did not find any evidence that the form of DIN available affected the composition of the phytoplankton community during the short duration of our experiment. Rather, contrary to previous studies of the SFBD, we found that in situ phytoplankton, both large-celled diatoms and small-celled cyanobacteria, in the lower Sacramento River are poised to bloom under in situ

nutrient conditions, if given sufficient light. We conclude that high anthropogenic NH_4^+ loading is not a driver of the lower productivity and pelagic organism decline in the SFBD.

Chapter 6

The response of San Francisco Bay phytoplankton photosynthetic yield to changes in dissolved inorganic nitrogen availability, temperature and season⁸

ABSTRACT

Over the last several decades, the northern San Francisco Bay-Delta ecosystem has witnessed declines in primary productivity. Understanding the causes of this low productivity, despite high nutrient availability, is of central ecological and societal concern. The phytoplankton populations in the northern portions of the San Francisco Bay-Delta system around Suisun Bay exhibit consistently low photosynthetic yield; the ratio of variable fluorescence to maximal fluorescence, indicating poor phytoplankton physiological state in this region. One hypothesis advanced for the cause of low productivity in this region is the suppression of phytoplankton nitrate uptake and growth due to high concentrations of wastewater-derived NH_4^+ . Here, we present one year of high-frequency dissolved inorganic nitrogen and in situ fluorescence measurements from underway sampling conducted as part of regular water quality monitoring cruises in San Francisco Bay. To our knowledge, this represents the first time in which simultaneous measurements of surface NH_4^+ , NO_3^- , and phytoplankton variable fluorescence (Fv/Fm) were made contemporaneously as continuous underway measurements. We find that high wastewater derived NH_4^+

⁸ A version of this chapter is written with Matt Mills, Raphe Kudela, Jim Cloern, Tara Schraga, Stephen Monismith, Chris Francis and Kevin Arrigo and is being prepared for submission for publication in *Estuarine, Coastal and Shelf Science*.

loading to the region is not the cause of the low Fv/Fm in Suisun Bay. We also find that the variability in surface photosynthetic yield in San Francisco Bay is driven primarily by changes in salinity, temperature, chlorophyll *a* concentration and suspended particulate matter concentrations, rather than nutrient availability, with seasonal variation in the dominant predictors. High-frequency NH₄⁺ measurements also revealed several hotspots of high NH₄⁺ concentrations missed by discrete sampling.

INTRODUCTION

Globally, humans now generate more fixed nitrogen (N) than do non-human biogeochemical processes (Vitousek et al. 1997; Gruber and Galloway 2008). The increase in the magnitude of anthropogenic fixed N delivery to coastal ecosystems from a variety of human activities is a principal cause of eutrophication and declines in ecosystem health around the planet (Townsend et al. 2003; Howarth et al. 2008). Many of these problems are linked to the responses of phytoplankton productivity and community composition to abundant fixed N, responses that can be variable and heterogeneous. Our detailed understanding of the fate of anthropogenic N and its ecological consequences in estuarine systems is constrained by data availability regarding the spatial and temporal dynamics of delivery and processing of DIN forms in coastal systems (Nixon et al. 1995). Such measurements form the basis of both our experimental and our model-based understanding of estuarine ecosystem processes. Increasing the spatial and temporal frequency of measurements of inorganic N loads to coastal systems is a key goal for ecologists and water quality managers that seek to

understand the dynamics and sources of biogeochemical variability in these systems and to define numeric water quality criteria to address nutrient loading that impairs ecosystem functioning. This scientific goal is especially salient in the San Francisco Bay ecosystem, which exhibits high concentrations of both anthropogenic nitrate (NO_3^-) and ammonium (NH_4^+), but low overall levels of phytoplankton productivity (Cloern and Jassby 2012).

San Francisco Bay and SF Bay-Delta

San Francisco Bay is the largest estuary on the west coast of North America. The bay is formed by two sub-estuaries with different hydrological dynamics: the northern portion of the Bay is a freshwater-to-saltwater, partially-mixed estuary that is dominated by inflows from the Sacramento and San Joaquin Rivers. These two rivers form the Sacramento-San Joaquin River Delta that empties into Suisun Bay at the northern end of the estuary. The southern portion of San Francisco Bay, which extends from the Golden Gate Bridge to the southern end of the Bay at Alviso in San Jose is a lagoonal estuarine system with low freshwater inputs and a narrower salinity range (salinities always > 20). Both the mixed estuarine system and the lagoonal system are characterized by high turbidity (Conomos et al. 1985). We refer to the San Francisco Bay, Suisun Bay, and Sacramento-San Joaquin River as the San Francisco Bay-Delta system (SFBD).

Since the mid-1980s, the northern reaches of SFBD have exhibited significant declines in phytoplankton production, particularly in Suisun Bay and the Delta (Cloern and Jassby 2012). The causes of the decline in phytoplankton productivity, despite

high ambient nutrient concentrations, have remained a subject of scientific debate and discussion for several decades (Alpine and Cloern 1992; Thompson 2005; Dugdale et al. 2007; Cloern and Jassby 2012; Strong et al. *under review*). One hypothesis advanced to explain the decline in northern San Francisco Bay productivity suggests that high inputs of anthropogenic NH_4^+ from the Sacramento Regional Wastewater Treatment Plant (SRWTP) effluent outfall, located on the Sacramento River, has caused declines in primary production in the LSR (the portion of the Bay Delta that is part of the Sacramento system) and Suisun Bay (Glibert 2010; Glibert et al. 2014). Other work has suggested that light limitation (Alpine and Cloern 1992) or grazing by the invasive clam *Potamocorbula amurensis* (Thompson 2005) have contributed to the decline in phytoplankton growth.

Recent work in the region has also demonstrated that the ratio of variable fluorescence to maximal fluorescence (F_v/F_m) of in situ phytoplankton populations, a measure of the photosynthetic performance of photosystem II, is very low in the Suisun Bay region. F_v/F_m values generally range from 0.0 (dead cells) to 0.7 (healthy cells conducting maximal photosynthesis). Although F_v/F_m will also vary with changes in phytoplankton community structure (Suggett et al. 2009), aquatic scientists widely use F_v/F_m as an indicator of phytoplankton physiological state, nutrient availability and health within an ecosystem (Saeck et al. 2016). The observed low photosynthetic yields in the northern SFBD are referred to as the “Bad Suisun” phenomenon (Kudela 2014). Measurements of F_v/F_m made in 2012 and 2013 have shown a hot-spot of “low F_v/F_m ” values located in Suisun Bay (Kudela 2014). Whether or not high anthropogenic wastewater NH_4^+ loads to this turbid estuary play a

role in the poor in situ photosynthetic performance of phytoplankton in the region is a salient and open scientific question.

Here we present one year of high-frequency DIN measurements from underway sampling conducted as part of regular water quality monitoring cruises in San Francisco Bay in 2014-2015 in order to assess the response of SFBD phytoplankton physiological state to changes in DIN availability and seasonality and to assess the relationships of productivity with temperature and salinity in a drought year. To our knowledge, our study represents the first time in which simultaneous measurements of surface NH_4^+ , NO_3^- , and F_v/F_m were made contemporaneously as continuous underway measurements in an estuarine system. We focus our attention on the “Bad Suisun” phenomenon, and also use our unique combination of assembled data sets to assess the drivers of variability in surface photosynthetic yield throughout the SF Bay system.

METHODS

Cruises

Monthly water quality sampling cruises in San Francisco Bay have been conducted by the United States Geological Survey (USGS) Water Resources Division since 1967. Here, we report under-way data from monthly full San Francisco Bay USGS Water Resources cruises on the *R/V Polaris* on which both a high-frequency NH_4^+ analyzer (FIALab 1500, Bellevue, WA) and a PhytoFlash Submersible Active Fluorometer (Turner Designs, San Jose, CA, USA) were deployed from May 2014 to May 2015. Additionally, an optical NO_3^- sensor (In-Situ Ultraviolet

Spectrophotometer (ISUS) Satlantic Inc, Halifax, NS) was also deployed from November 2014-April 2015.

The monthly cruise track runs along the central ship channel of San Francisco Bay from a station in LSB(the exact location depends on the tidal range that month which can limit ship access to portions of Lower South Bay) to a station in the LSR near Rio Vista, CA, a distance of just under 150 km (Fig. 6-1). All cruise sampling took place between 05h00 to 18h30, with the time of each monthly cruise track dependent on tidal currents and ship speed. Most measurements were made during daylight hours.

Under-way Measurements

Additionally, under-way water quality measurements were made using near-surface (intake at 2 m depth) water, continuously pumped through the flow-through seawater system of the *R/V Polaris*. All under-way measurements (Chl *a*, SPM, Fv/Fm, salinity, temperature, NH_4^+ , and NO_3^-) were made on the same flow-through system.

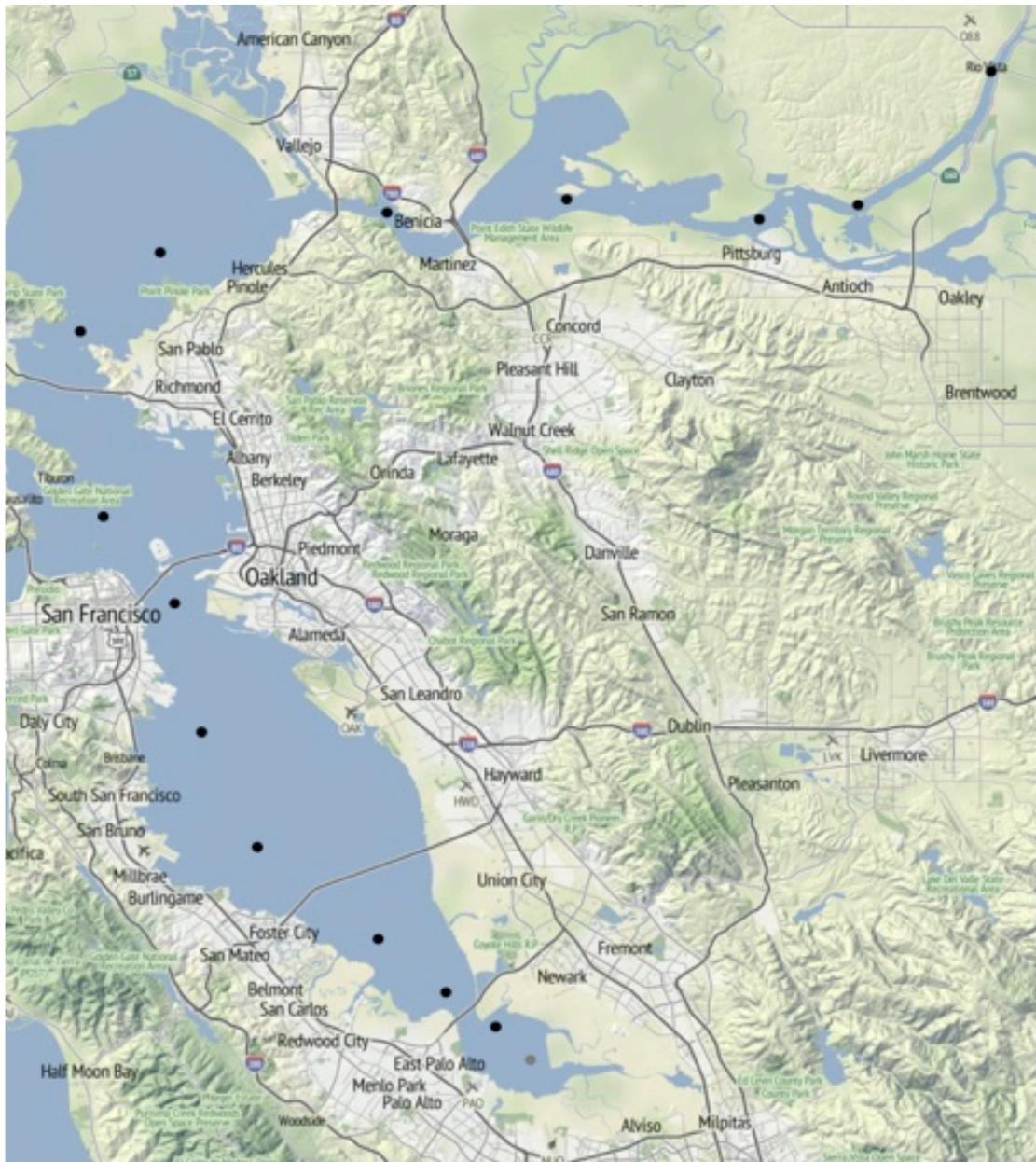


Figure 6-1. Regular discrete sampling stations (shown as black dots) along the USGS monthly water quality monitoring cruise track in SF Bay. Station 36 (in gray in Lower South Bay) is the start point for most transects, which end in Rio Vista, CA in the Sacramento River.

High-Frequency Ammonium Measurements

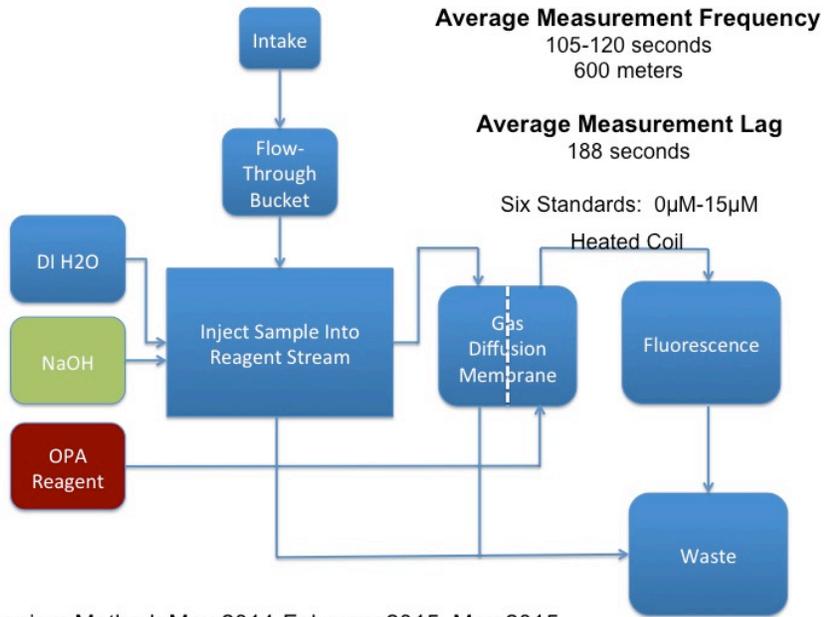
From the May 2014 cruise to the May 2015 cruise, near-surface water from the ship's flow-through system was pumped through darkened 1.25 cm ID tubing to an

open 1 L high-density polyethylene “bucket” (Nalgene®), at a rate of $\sim 4.0 \text{ L min}^{-1}$. Water in the sample bucket was then sampled for NH_4^+ concentration using a custom-designed, field-deployable NH_4^+ analyzer. This flow-injection analysis-gas-diffusion (FIA-GD) system was adapted to employ the wet-chemical method of Holmes et al. (1999), and uses fluorometric development after reaction with ortho-phthaldialdehyde (OPA). Briefly, water was pumped continuously from the chamber to the instrument through a 0.125 in ID tube using a peristaltic pump. At regular intervals, sample water was injected into a continuous flow carrier (sterile, de-ionized H_2O), and mixed with 20 mM NaOH to increase pH, converting all NH_4^+ in the sample water to NH_3 . The water was then pumped along a gas-permeable Teflon™ membrane, which allowed any NH_3 present to diffuse across the membrane into a stream of fluorometric development OPA reagent containing ortho-phthaldialdehyde (1.6 g L^{-1}), sodium tetraborate decahydrate (20 g L^{-1}), and sodium sulfite (0.24 g L^{-1}) pumped in the opposite direction. The reagent- NH_3 mixture was then pumped through tubing wrapped around a heated (67°C) coil to increase the rate of development and measured fluorometrically. Figure 6-2A shows a diagram of the flow path for analysis. Surface NH_4^+ measurements were made every 105-120 s (depending on the speed at which the peristaltic pump was operated), while under-way. Fluorometric peaks were converted to time-stamped NH_4^+ concentrations using a six-point standard curve (0, 0.47, 0.94, 1.88, 3.75, 7.5, and $15 \mu\text{M NH}_4^+$ with regular standard and blank checks). Standard curves had R^2 values > 0.9998 . Analytical precision, based on replicate analyses, was $\leq \pm 6\%$.

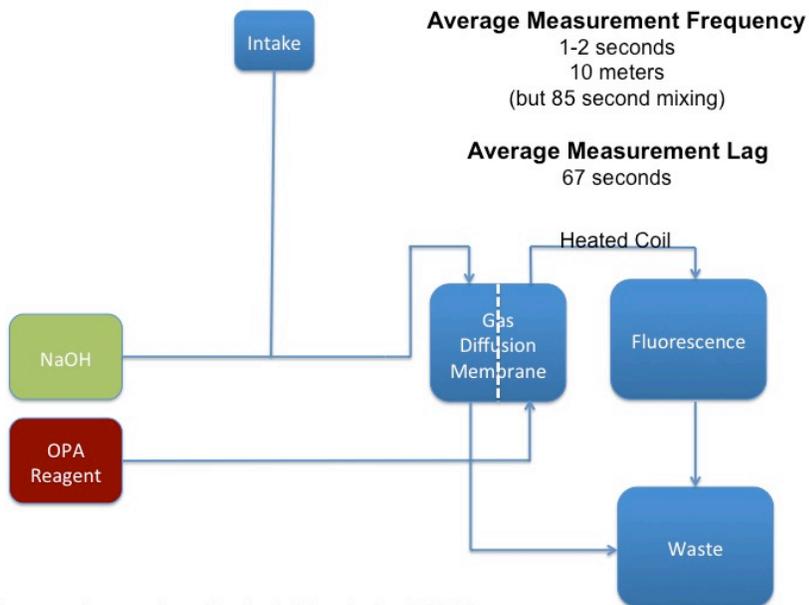
During cruises in March and April 2015, an alternative configuration of the flow-through system was used. Rather than injection of sample water into a carrier stream, the analyzer was re-plumbed so that sample water was continuously pumped and mixed with 20 mM NaOH and passed along a gas diffusion membrane, across which sample NH_3 diffused into a clean stream of the same OPA reagent described above. Thus, rather than a series of peaks corresponding to regular discretized sample injections, the fluorometric analysis produced a continuous trace. Fluorometric counts were reported at 0.75 Hz. Sample curves were generated by pumping through the series of six standards in succession, generating a “step-function” increase on the trace, which enabled fluorometric counts to be converted to estimates of concentration. A diagram of this configuration is shown in Figure 6-2B.

High-Frequency Nitrate Measurements

Prior to sampling from a flow-through bucket for analysis of NH_4^+ , near-surface flow-through water was also pumped through an ISUS equipped with a flow-through cap. This sensor measured NO_3^- concentrations at 1 s intervals at a detection limit of 2 μM NO_3^- .



Ammonium Method: May 2014-February 2015, May 2015



Continuous Ammonium Method: March-April 2015

Figure 6-2 Schematic design of NH₄⁺ analyzer in sample injection mode (A) and in continuous sample trace flow mode (B).

Fv/Fm Measurements

The quantum yield of photosystem II is given by F_v/F_m , the ratio of variable fluorescence (F_v) to maximal fluorescence (F_m). For in-situ fluorometers, F_v is calculated as the difference in fluorescence between in situ fluorescence (F_o') without a saturating light and the fluorescence after cells are exposed to a saturating irradiance (F_m'). On each monthly full-bay cruise transect, a blue actinic-light (460 nm) LED PhytoFlash (Turner Designs, San Jose, CA) was connected to the ship's under-way flow-through water system. F_v/F_m was measured at 1-minute intervals throughout each cruise track.

Chlorophyll Fluorescence Measurements

Near-surface flow-through water was pumped through a 10-AU Field Fluorometer (Turner Designs, San Jose, CA), which measured Chl *a* fluorescence every five seconds, producing output in voltage from the photomultiplier tube. Fluorometer voltage measurements were converted into Chl *a* concentrations by linear regression with discrete near-surface Chl *a* samples taken on each cruise. Following the approach used by USGS Water Resources for water-column fluorometer measurements, linear regressions of fluorometer voltage outputs and Chl *a* were performed for each monthly cruise. If a linear regression of all data for the full sampling transect yielded a poor model fit ($R^2 < 0.7$) with high standard error, additional linear regressions were repeated for sub-sets of transect data (separated by embayment), or multiple linear regressions including terms for turbidity and salinity measurements were included, until regression fit standard error was $< 5\%$. Regression

parameters (slope and intercept) were used to convert voltage units into concentration units for all data.

Suspended Particulate Matter Measurements

Near-surface flow-through water was also pumped through an Optical Backscatter Reflectometer (Turner Designs, San Jose, CA), which measures particle absorption and backscatter every five seconds, producing estimates of water turbidity, output in voltage units. Turbidity measurements were converted from voltage into estimates of suspended particulate matter concentration by linear regression with near-surface discrete suspended particulate matter (SPM) (mg L^{-1}) samples measured gravimetrically and taken at 14 stations. Separate linear regressions of SPM to turbidity voltage were performed for each monthly cruise transect. Regression parameters (slope and intercept) were used to convert voltage units into concentration units for all data. Unlike for Chl *a*, a single linear regression was performed for SPM for each full cruise track and all R^2 values were > 0.8 .

Additional Under-Way Measurements

The temperature and salinity of near-surface water pumped through the ship's flow-through system was also recorded every 5 seconds while under way.

Temperature was measured to the nearest 0.1°C and salinity was measured to an accuracy of 0.01 using a multi-point calibration. It should be noted that the calibration file was incorrectly loaded during June 2014, resulting in a loss of both salinity and temperature data during this monthly cruise.

Mapping Data

We converted time-stamped underway measurements to spatial coordinates using the ship's GPS log, with ship location geotagged and logged at 5 s intervals. Because underway data analysis was nearly instantaneous for Chl *a*, SPM, salinity and temperature, no time-lag correction was applied for these variables, and those data were mapped to the location of the ship at the time of sample measurement. For NO₃⁻ underway measurements, a time lag of 45 seconds was applied, to account for the time it took water to pass through the ship's flow-through system to reach the NO₃⁻ sensor. For NH₄⁺ underway measurements, a time lag of 188 seconds was used, to account for the time required to pump water from the flow-through system into the analyzer and to perform the fluorometric development and analysis of the concentration. For the continuous flow trace analysis used in March and April 2015, this lag was 67 seconds.

Regional Analyses

Consistent with Novick and Senn (2014) and management practice for the regions, we separated the San Francisco Bay into five sub-embayment regions to analyze the relationships of different environmental parameters: Lower South Bay (LSB), South Bay (SB), Central Bay (CB), San Pablo Bay and Carquinez Strait (SPB), and Suisun Bay (SUI) (from Carquinez Strait to the Bay Delta). To these five regions, we added the Lower Sacramento River (LSR), from the confluence of the San Joaquin and Sacramento Rivers to Rio Vista, CA), which was also included in our study area for a total of six study regions (Fig. 6-3).



Figure 6-3 Six regional designations for separation and analysis of different sub-embayments.

Data and Regression Analysis

All under-way datasets were combined for each cruise into a single file.

Because temporal sampling frequencies varied between different measurements (Chl *a*, SPM, salinity, temperature at 5 s; FvFm at 60 s, NO₃⁻ at 1 s and NH₄⁺ at either 120

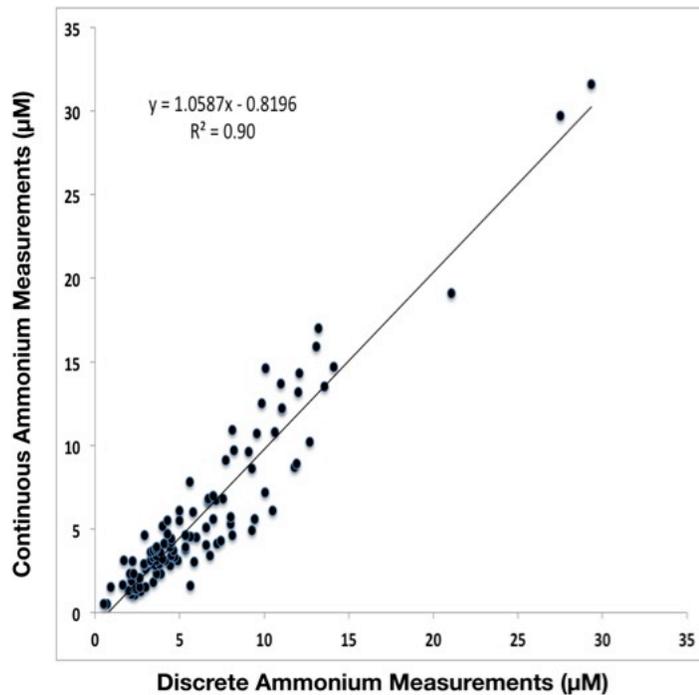
or 0.75 s, depending on method), data were interpolated to a uniform time-interval of 5s. All data analyses were performed in R (version 3.0.2, September 2013).

In order to assess the response of Fv/Fm to measured environmental drivers (salinity, temperature, NH_4^+ , NO_3^- , SPM, and Chl *a*), we performed multiple linear regression analysis and calculated relative importance values – an estimate of the relative contribution in % of the total explanatory power of each predictor -- using the **relaimpo** package in R. We performed this analysis for both the full cruise-track dataset for each cruise, and for a data subset that combined the SUI and LSR regions. For some regions in some years, environmental predictor variables exhibited varying degrees of multicollinearity, with calculated variance inflation factors (vif) >10. This was not the case for all variables in all months, but due to the potential sensitivity of results to the correlation of predictor variables with each other, we performed an additional set of analyses using partial least squares regression, using the **pls** package in R, using two components. We then calculated the variance in Fv/Fm explained by each of the two components and performed factor analysis to assess the contributions of environmental variables to each component.

Performance of Underway DIN Instrumentation

In order to assess the accuracy of the underway NH_4^+ and NO_3^- analyzers, we regressed all discrete surface DIN measurements made over the 13 months against the contemporaneous measurement of DIN from the underway analyzer. The time stamp recorded for filling the discrete bottle was used to determine the corresponding underway measurement.

Both of the continuous DIN instruments performed well for *in situ* environmental samples, $R^2 = 0.9$ (Fig. 6-4AandB). Regressions of discrete vs. continuous measurements for both instruments had slopes slightly greater than 1 and the NO_3^- analyzer had a significant negative intercept, indicating the potential need for offsetting concentrations, especially at low NO_3^- values.



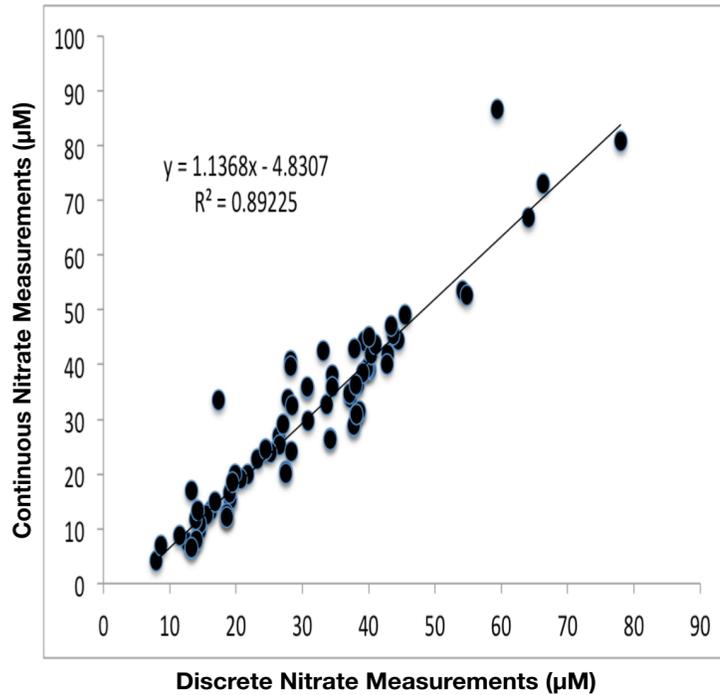


Figure 6-4 Regression analysis of discrete vs. continuous measurements for NH₄ analyzer (A) and NO₃ sensor (B).

RESULTS

Spatial and Seasonal Patterns

Salinity and Temperature

Salinity was greatest in summer and fall, peaking in October, and lowest from January to April, due both to increases of freshwater input in the winter rainy season and lower mean temperatures. From CB to the LSR, the San Francisco Bay system is an estuary with a strong salinity gradient from almost full-salt seawater (salinity > 30) to freshwater (salinity < 2). Salinities along the estuarine gradient were always greatest near in CB and lowest in LSR. In the southern portion of the Bay, salinities were greater than in CB during the summer and fall months (July through November)

due to evaporation, but lower than in CB during the winter and spring (December through May) due to freshwater inputs. Surface water temperature followed a seasonal cycle, peaking at a mean of 21.2°C in August 2015 and declining to 12.2°C in January.. The lowest maximal temperatures were in CB, where monthly mean water temperatures never exceeded 19.5°C, and the greatest summer maximal temperatures were in LSB (>22°C in August) and LSR (>23°C in July). In winter, the lowest minimum temperatures were observed again in the LSR (<10.5°C in January), but only dropped to 13°C in CB. Thus, due to strong terrestrial influences, the LSR exhibits a much greater range in surface water temperatures seasonally than CB.

Ammonium

The highest NH_4^+ concentrations were always found in the LSR region (mean across all months: $9.2 \pm 5.6 \mu\text{mol L}^{-1} \text{NH}_4^+$), significantly greater than for all other regions ($p < 0.05$). NH_4^+ concentrations in CB, South Bay and LSB were more variable, occasionally reaching concentrations as high as 10-15 $\mu\text{mol L}^{-1}$ or concentrations as low as 0.5 $\mu\text{mol L}^{-1}$. Yearly mean values for these two regions were approximately the same (3.7 ± 2.0 and $3.4 \pm 1.4 \mu\text{mol L}^{-1}$), and were significantly lower ($p < 0.05$) than concentrations in SPB (mean= $4.3 \pm 1.2 \mu\text{mol L}^{-1}$) or SUI (mean= $5.7 \pm 4.1 \mu\text{mol L}^{-1} \mu\text{M}$) (Figure 6-5).

The high NH_4^+ concentrations in the LSR originated at the sampling terminus in Rio Vista, CA. Concentrations declined along a mixing curve in an identical spatial pattern, repeated across all sampling months regardless of the magnitude of the concentrations. Monthly patterns of NH_4^+ concentration in the LSR were similar across all months (Fig. 6-6). Plotting these mixing curves in relation to changes in temperature and salinity demonstrates that concentrations are changing not due to changes in water masses or additional inputs, but due to the mixing of the high NH_4^+ Sacramento River water with a water mass with lower NH_4^+ from San Joaquin and SUI waters in this region (Figure 6-7). During May 2014-May 2015, there was a strong seasonal cycle in NH_4^+ concentrations in the LSR and SUI. NH_4^+ concentrations were lowest from May to October and greatest in the winter and spring, from November to April.

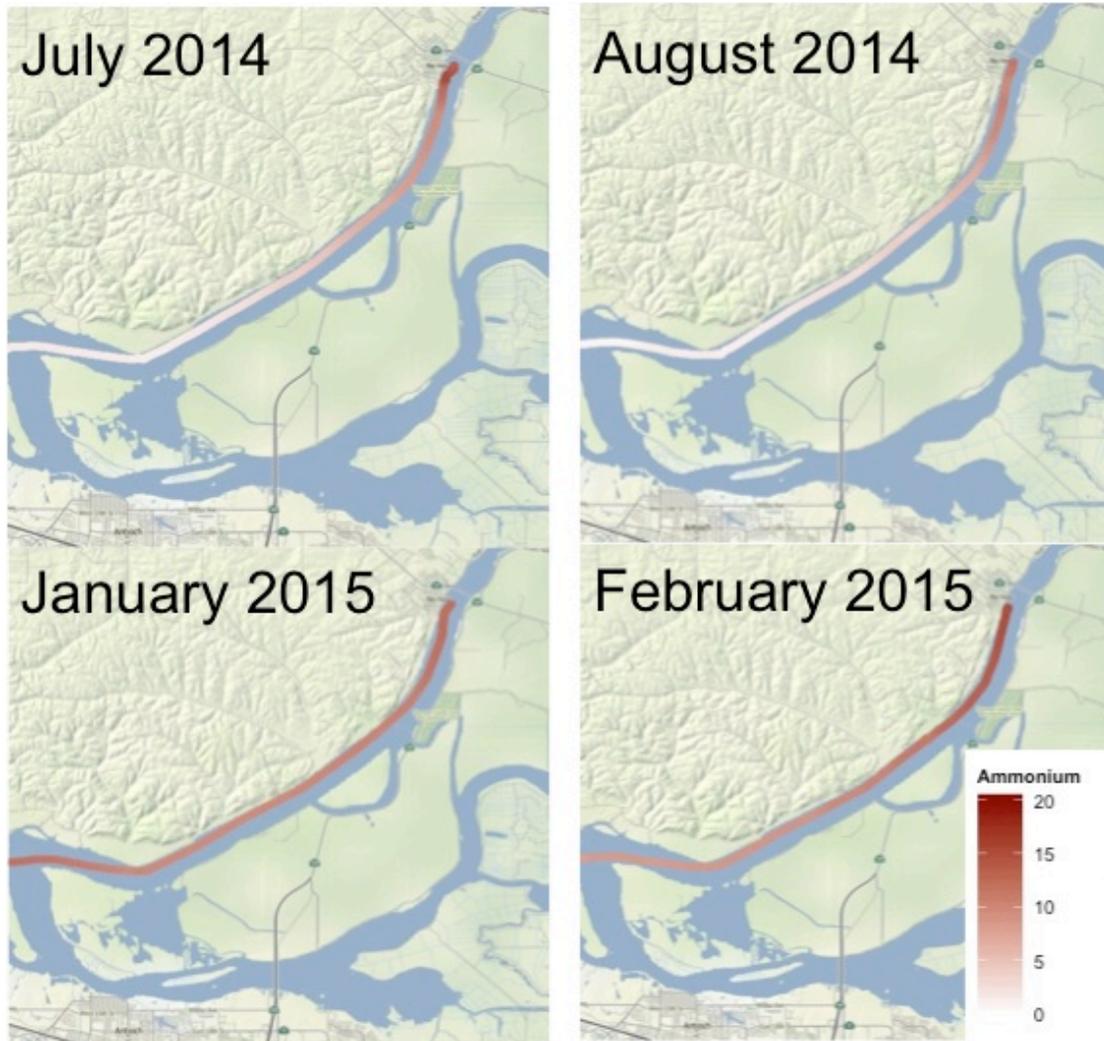


Figure 6-6 NH₄⁺ concentrations decline heading downstream in LSR across all seasons.

Nitrate

Average surface NO₃⁻ concentrations were uniformly high in LSB (mean values 40-80 μmol L⁻¹), SB (25-40 μmol L⁻¹), SUI (25-42 μmol L⁻¹), and the LSR (32-50 μmol L⁻¹ NO₃⁻), and relatively low in CB (7-15 μmol L⁻¹). SPB, located between SUI and CB, had intermediate values (means ranging 14-22 μmol L⁻¹). The lowest value for NO₃⁻ recorded was 3.7 μmol L⁻¹ in CB in March 2015. The highest value recorded was 80.9 μmol L⁻¹ in LSB in January 2015. This range mirrors the range of

discrete values over the same time period, for which the lowest measured value was $7.4 \mu\text{mol L}^{-1}$ at Station 18 (CB) in March 2015 and the highest measured value was $78.0 \mu\text{mol L}^{-1}$ at Station 36 (LSB) in January 2015. NO_3^- was only measured on cruises from November to April, so no assessment of NO_3^- seasonality could be made.

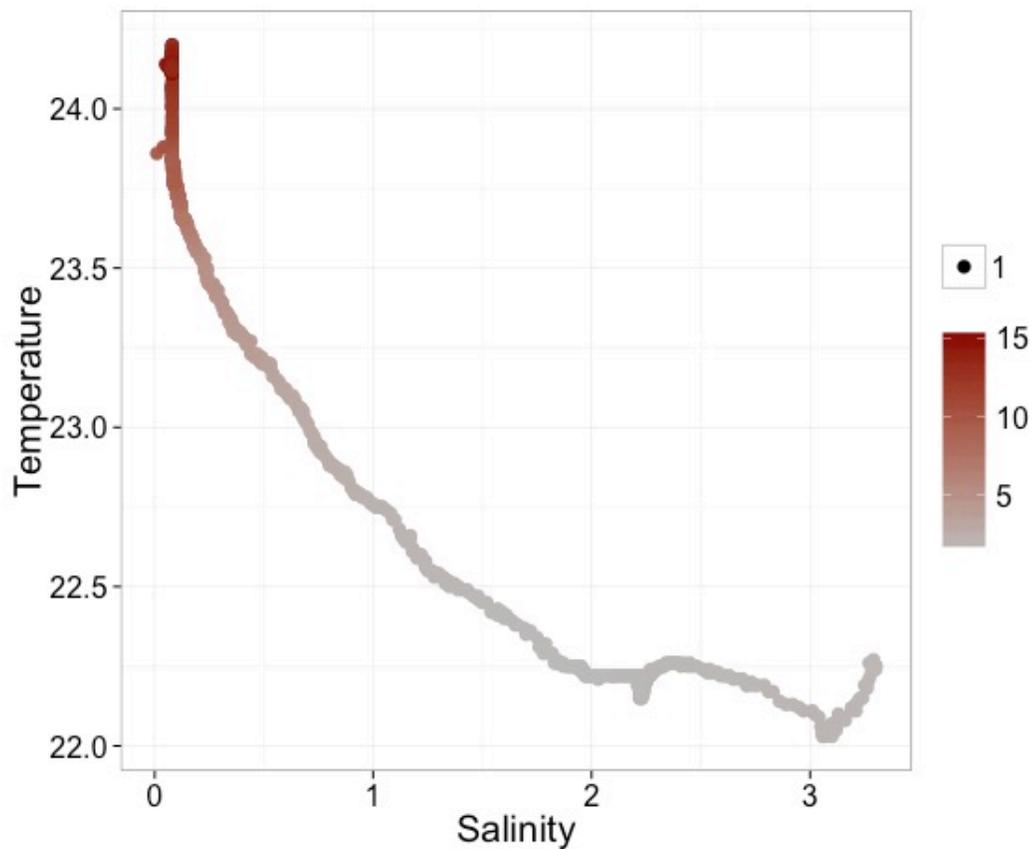
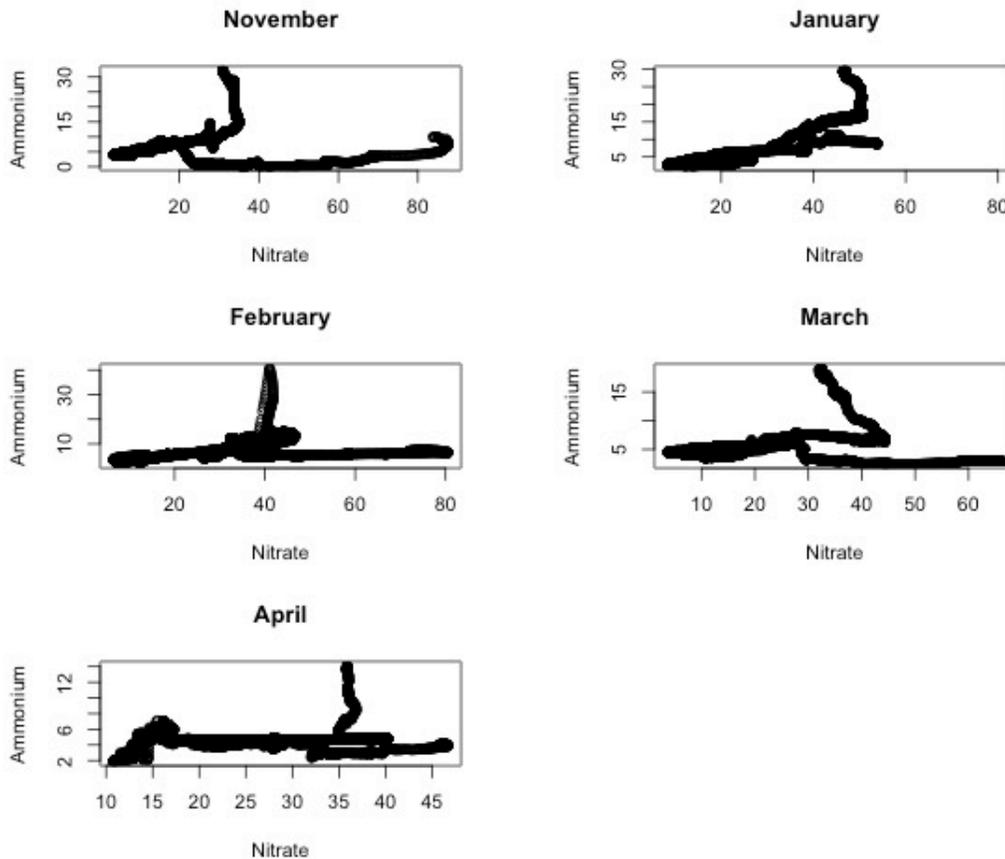


Figure 6-7. Temperature-Salinity diagrams July for LSR NH_4^+ .

Nitrification

By simultaneously measuring surface concentrations of both NH_4^+ and NO_3^- , we are able to assess whether any regions presented evidence of nitrification (the microbially oxidation of NH_4^+). We assessed whether any regions in any months

exhibited patterns of declining NH_4^+ with increasing NO_3^- concentrations. Across the five months, only the upper stretches of the LSR exhibited increasing NO_3^- concentrations and decreasing NH_4^+ concentrations (Fig 6-8A). For November, January, February and April cruises, the slopes of the decline in NH_4^+ relative to increase in NO_3^- were significantly greater than 1, indicating that nitrification, while it may be occurring, is not the only fate of NH_4^+ . However, in March in the LSR (Fig 6-8B), the slope is -0.95, very close to the 1:1 conversion of NH_4^+ to NO_3^- expected in nitrification.



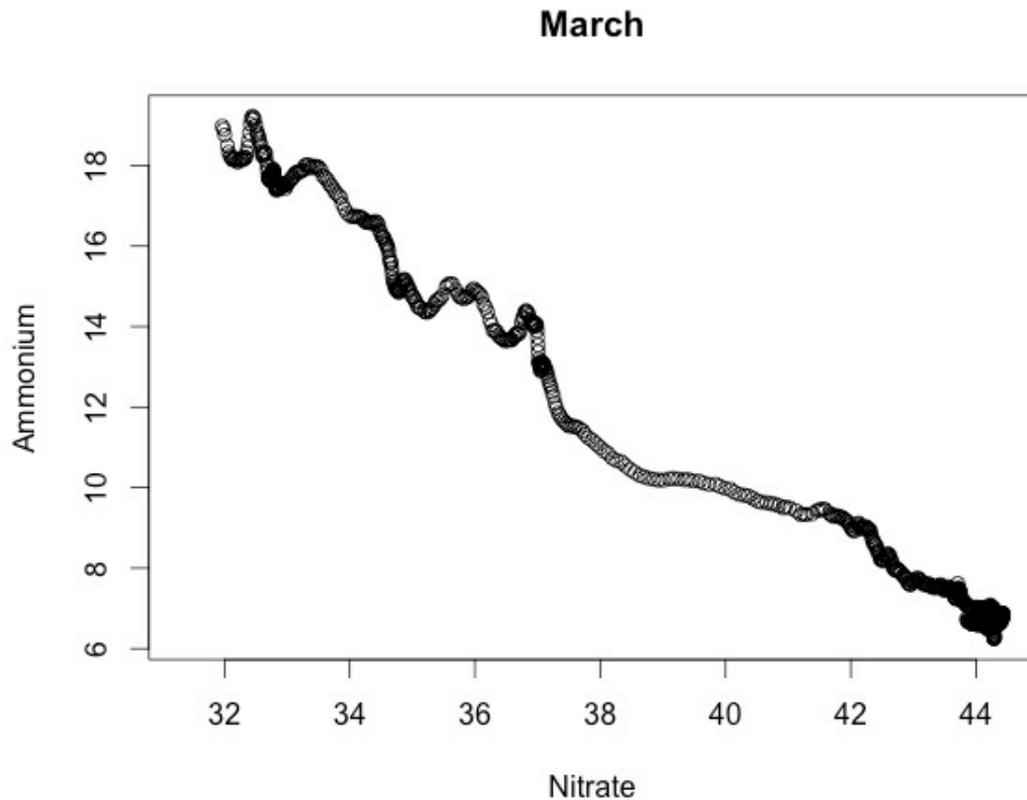


Figure 6-8. NO_3^- concentrations vs. NH_4^+ concentrations for five months of full cruise data (A). The regions of declining NH_4^+ and increasing NO_3^- are located in LSR for each month. In March 2015 the slope of NH_4^+ vs. NO_3^- in LSR was ~ 1.0 (B).

Suspended Particulate Matter (SPM)

Like NO_3^- and NH_4^+ , SPM concentrations were greatest at the ends of the sampling transect and lowest in the middle. SPM was always greatest in the Lower South Bay, which had a monthly average over $111 \pm 104 \text{ mg SPM L}^{-1}$ during our sampling year. The lowest SPM concentrations were found in CB, adjacent to the Golden Gate Bridge, which had a monthly average below 20 mg SPM L^{-1} . South Bay as a whole (monthly mean $72.9 \pm 71.9 \text{ mg SPM L}^{-1}$) was significantly more turbid than CB (monthly mean $19.6 \pm 15.0 \text{ mg SPM L}^{-1}$) or SPB (monthly mean $24.9 \pm 18.4 \text{ mg SPM L}^{-1}$).

SPM L⁻¹). SUI was marginally more turbid than SPB (monthly mean 36.5±17.5 mg SPM L⁻¹). The only exception to this trend was in July, when a strong westerly wind resulted in SPB being more turbid than SUI. The LSR exhibited similar turbidity to SUI as a whole. SPM was greatest in the spring and summer from April to August and lowest in winter. High July SPM was linked to high concentrations in San Pablo and SUI bays during typically windy summer months in these sub-embayments.

Chlorophyll a

The highest mean Chl *a* values along the transect were always measured in LSB. The annual maximum concentrations were measured during the spring phytoplankton bloom. In March 2014, and from March-May 2015, LSB underway Chl *a* concentrations averaged between 9.0±1.4 and 12.5±1.5 µg L⁻¹. Across all seasons, the regions with lowest average Chl *a* concentrations were both SUI and SPB. Chl *a* concentrations were always higher in SB and CB than in San Pablo and SUI (except during a March 2015 bloom in a portion of SUI). Similarly, Chl *a* concentrations were always greater in the LSR compared to SUI, and, when compared to SPB, with the exception of January and February. Thus, Chl *a* decreased from South Bay to CB, remained low through San Pablo and SUI bays, and increased again into the LSR (Figure 6-9).

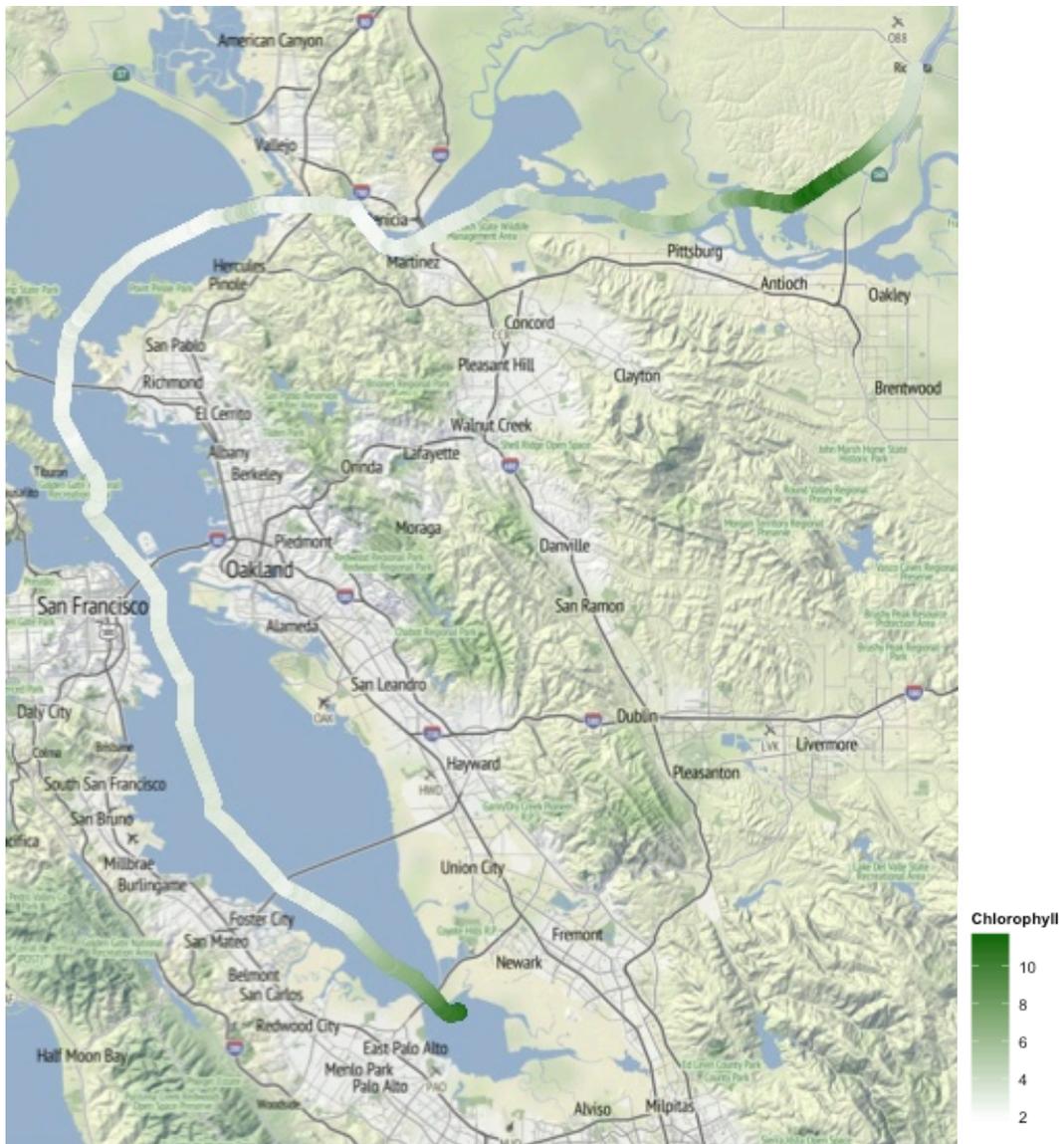


Figure 6-9 Chl *a* concentration in March 2015.

Chl *a* exhibited strong seasonal cycles, following a typical primary spring bloom – secondary fall bloom pattern. Concentrations were highest from March to June, then dropped during the summer, only to rise again in September. The lowest Chl *a* concentrations were observed during the winter months from November to February.

F_v/F_m

For most of the year, F_v/F_m was relatively constant across the full San Francisco Bay, with monthly means from 0.45 to 0.49 for all months except the winter months of January-March, when values were lower. For most of the year, the underway near-surface F_v/F_m indicates photosynthetically productive and healthy phytoplankton community throughout San Francisco Bay. The spatial pattern of F_v/F_m mirrored that of Chl *a*. F_v/F_m was greatest in LSB and South Bay, and lowest in SUI and portions of SPB and the LSR. Across all months, F_v/F_m averaged 0.56 ± 0.05 in South Bay and 0.51 ± 0.05 in CB, which was significantly greater than the yearly means for SPB (0.42 ± 0.03), SUI (0.36 ± 0.07) or the LSR (0.41 ± 0.12). Across all months, F_v/F_m consistently declined from south to north along the sampling transect, being greater in the South Bay lagoonal estuarine system than in the San Francisco Bay-Delta estuarine system.

Bay-Wide and Regional Response of F_v/F_m to Environmental Factors

The relationship of environmental conditions and the physiological state of phytoplankton, as measured by F_v/F_m , was assessed for all San Francisco Bay data for each monthly cruise using a combination of relative importance values calculated from multiple linear regression and from partial least squares regression. We repeated these analyses for the subset of data from SUI and the LSR (Tables 6-1 and 6-2). Overall, the explanatory power of multiple regression models was high. For the full Bay multiple regression models, environmental and biological variables explained between

38.3% and 96.7% of the variance in Fv/Fm. Model performance was especially strong during January-April 2015 ($R^2 > 0.90$ for all months).

Table 6-1. Full Bay The relative importance of environmental variables in predicting observed Fv/Fm surface data collected throughout the SF Bay transect. The % of variance in Fv/Fm explained by the linear model and the estimated coefficients for each predictor are also reported.

Relative Importance of Predictor	5/14	6/14	7/14	8/14	9/14	10/14	11/14	1/15	2/15	3/15	4/15	5/15
Temperature	0.07	NA	0.12	0.09	0.08	0.08	0.38	0.20	0.22	0.16	0.08	0.27
Salinity	0.35	NA	0.45	0.11	0.35	0.13	0.06	0.18	0.23	0.13	0.21	0.41
Ammonium	0.25	0.18	0.02	0.12	0.04	0.08	0.06	0.12	0.12	0.10	0.02	0.04
SPM	0.03	0.15	0.12	0.08	0.13	0.70	0.20	0.17	0.20	0.12	0.11	0.11
Chlorophyll	0.30	0.67	0.29	0.59	0.40	0.01	0.18	0.21	0.14	0.38	0.41	0.15
Nitrate	NA	NA	NA	NA	NA	NA	0.13	0.12	0.09	0.14	0.16	NA
% of Variance Explained by Model	96.74	73.87	86.58	80.53	38.29	68.36	55.59	95.94	95.78	90.67	92.91	79.18
Full Model Coefficients	5/14	6/14	7/14	8/14	9/14	10/14	11/14	1/15	2/15	3/15	4/15	5/15
Temperature	-0.065	NA	0.006	-0.009	-0.13	-0.04	0.054	0.15	0.095	-0.05	-0.01	0.03
Salinity	-0.052	NA	0.007	0.0006	0.003	-0.004	0.0007	-0.004	0.008	-0.007	0.005	0.01
Ammonium	0.003	0.14	-0.002	0.007	-0.003	0.004	0.004	0.0001	-0.0009	0.006	0.01	0.009
SPM	-0.0008	-0.0004	-0.001	-0.00008	0.005	-0.007	0.0006	-0.0005	0.0003	0.002	-0.001	-0.0009
Chlorophyll	0.04	0.06	0.08	0.04	0.14	-0.002	0.05	0.04	0.003	0.03	0.04	0.05
Nitrate	NA	NA	NA	NA	NA	NA	-0.0003	0.0009	-0.0003	-0.007	-0.002	NA

Table 6-2. Suisun Bay and Lower Sacramento River. The relative importance of environmental variables in predicting observed Fv/Fm surface data collected in Suisun Bay. The % of variance in Fv/Fm explained by the linear model and the estimated coefficients for each predictor are also reported.

Relative Importance of Predictor	5/14	6/14	7/14	8/14	9/14	10/14	11/14	1/15	2/15	3/15	4/15	5/15
Temperature	0.07	NA	0.11	0.20	0.01	0.02	0.26	0.34	0.24	0.01	0.10	0.02
Salinity	0.35	NA	0.05	0.12	0.52	0.51	0.16	0.14	0.27	0.24	0.10	0.02
Ammonium	0.25	0.33	0.20	0.23	0.14	0.05	0.22	0.07	0.01	0.12	0.07	0.19
SPM	0.03	0.16	0.06	0.05	0.23	0.39	0.06	0.08	0.23	0.12	0.10	0.05
Chlorophyll	0.30	0.51	0.58	0.39	0.09	0.02	0.12	0.21	0.23	0.37	0.41	0.72
Nitrate	NA	NA	NA	NA	NA	NA	0.17	0.16	0.02	0.14	0.23	NA
% of Variance Explained by Model	96.74	72.04	89.02	91.34	38.98	54.00	84.16	95.64	94.49	95.14	91.23	74.11
Full Model Coefficients	5/14	6/14	7/14	8/14	9/14	10/14	11/14	1/15	2/15	3/15	4/15	5/15
Temperature	-0.065	NA	0.050	0.046	-0.0027	0.023	-0.040	0.196	0.010	-0.0009	-0.014	-0.024
Salinity	-0.052	NA	0.0088	0.0032	-0.0026	-0.0026	0.0006	0.002	0.007	-0.011	0.0036	0.0003
Ammonium	0.003	0.009	-0.002	0.0029	0.00003	-0.0014	0.0007	0.004	-0.001	-0.00008	0.008	0.02
SPM	-0.0008	0.0003	-0.0001	0.00005	0.00096	0.0024	-0.0006	-0.0004	-0.001	0.0017	-0.001	-0.002
Chlorophyll	0.042	0.053	0.072	0.025	0.019	-0.0023	0.047	-0.043	0.22	0.026	0.037	0.21
Nitrate	NA	NA	NA	NA	NA	NA	0.005	0.009	0.003	-0.008	-0.007	NA

The most important driver of Fv/Fm variability was not constant across all months. Seasonal trends, however, were present. From May 2014 to September 2014, Chl *a* concentration and salinity exhibited strongest positive correlations with Fv/Fm. In October 2014, the month with the highest SPM concentrations, SPM was the strongest driver of Fv/Fm variability and SPM concentration was negatively correlated with Fv/Fm. From November 2014 until March 2015, temperature was the strongest driver of Fv/Fm variability, contributing up to 38% of the explained variance in November 2014. NH_4^+ and NO_3^- concentrations played minor roles in explaining Fv/Fm variability across SF Bay.

These same patterns were observed in SUI/LSR. In this system, environmental and biological predictors explained 38-97% of the variance in Fv/Fm, and model performance was again strongest in January – April 2015. Salinity and SPM were the strongest predictors of Fv/Fm variability in September and October 2014, while temperature was the strongest control in November 2014-February 2015. Notably, given the presence of a spring bloom in this region in 2015, Chl *a* concentration was the most important predictor of Fv/Fm variability in March, April and May 2015.

Partial least squares regression analysis also was used to identify two new components that explain variation in Fv/Fm (Tables 6-3 and 6-4). In partial least square regression, like in principal component analysis, components are unitless latent variables and the measured predictors contribute to varying degrees to their variation. No consistent set of predictors were identified that contributed to the variation in Fv/Fm explained by the two components (in other words, no specific set of variables was clearly driving Fv/Fm variability). However, loading analysis demonstrated that

salinity and SPM concentrations consistently played the largest role in determining the variation of Fv/Fm.

Table 6-3 Two component partial least squares regression for Full Bay, showing factor analysis weights for each component and % variance explained by each component.

Variance Explained in Fv/Fm by 2 components in PLS Regression	5/14	6/14	7/14	8/14	9/14	10/14	11/14	1/15	2/15	3/15	4/15	5/15
Component #1	15.21		13.97	41.43	19.52	46.73	34.54	82.36	73.77	8.71	11.14	22.53
Component #2	54.20		49.38	2.54	13.22	9.80	4.80	7.18	11.23	58.75	36.21	50.07
LOADINGS Component #1	5/14	6/14	7/14	8/14	9/14	10/14	11/14	1/15	2/15	3/15	4/15	5/15
Temperature												
Salinity			0.121	0.685	0.994	-0.880		0.402	0.315	-0.509		
Ammonium	0.353					0.149						
SPM	-1.724		1.060	-0.730	-0.385	-0.625	0.708	-0.696	-0.904	0.587	1.193	1.008
Chlorophyll					0.100	-0.109						
Nitrate							0.746	-0.546	-0.301	0.692		
LOADINGS Component #2												
Temperature				-0.105						-0.148		-0.105
Salinity	-0.111		0.886	1.104		0.845	0.919	0.438	0.125	0.167	0.610	0.977
Ammonium	0.377				-0.123	-0.152	-0.374	-0.239				
SPM	0.923		-0.454	1.205	0.998	-0.524		-0.348	-0.198	0.586	-0.553	-0.176
Chlorophyll										0.297		
Nitrate							-0.250	0.902	1.128	-0.867	-0.577	

Table 6-4. Two component partial least squares regression for Suisun Bay and Lower Sacramento River showing factor analysis weights for each component and % variance explained by each component

Variance Explained in Fv/Fm by 2 components in PLS Regression	5/14	6/14	7/14	8/14	9/14	10/14	11/14	1/15	2/15	3/15	4/15	5/15
Component #1	15.21	16.61	3.31	26.20	30.96	47.19	49.92	41.04	82.16	59.25	38.46	1.416
Component #2	69.41	63.49	52.00	63.76	32.49	52.96	71.91	49.77	92.75	76.24	70.33	24.83
LOADINGS												
Component #1	5/14	6/14	7/14	8/14	9/14	10/14	11/14	1/15	2/15	3/15	4/15	5/15
Temperature												
Salinity			0.274	-0.412	-0.863	-0.920	-0.403	0.250	0.168	-0.444	-0.163	-0.484
Ammonium	0.353			0.111	-0.315	0.166	0.243					0.123
SPM	-1.724	1.022	1.400	-0.979	0.394	0.425	0.925	-0.915	-0.984	0.731	-0.992	-1.145
Chlorophyll										0.170		
Nitrate							0.264	-0.334		0.542		
Component #2												
Temperature			0.120	0.123	0.222							-0.162
Salinity	-0.111		-1.82	-0.781	-1.186	0.186	-0.307	1.073	0.592	-0.629	-1.013	1.400
Ammonium	0.377	0.968	0.692	0.201	1.581	-0.961	0.595	-1.060	0.255	0.560		0.542
SPM	0.923	-0.193	-0.692	0.750	-1.406	0.213	-0.741	0.937		-0.615	0.332	0.113
Chlorophyll		0.174		0.149	0.106						0.586	
Nitrate							0.125	-1.073	0.883	-0.130		

SUI and LSR Fv/Fm and the Ammonium Question

In January and February 2015 (Figure 6-10), mean Fv/Fm values were significantly lower in SUI and the LSR region (0.18 ± 0.06 and 0.26 ± 0.07 , for the two months respectively) than in any other month (mean Fv/Fm value for all other months: 0.46 ± 0.04). Outside of these two months, Fv/Fm was >0.35 in these two regions in all other months. Such values may indicate impairment of photosynthetic activity, but not complete inhibition of growth.

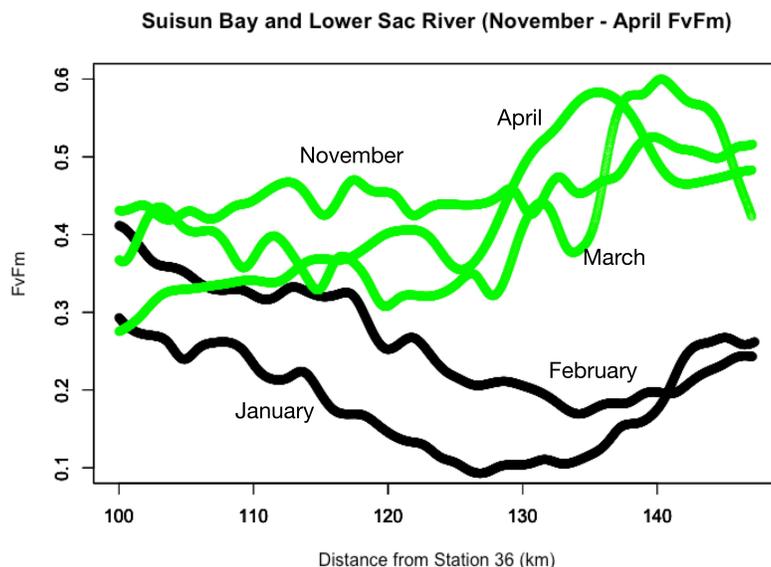


Figure 6-10 The sharp decline in Fv/Fm in the Suisun Bay/Lower Sacramento River region is shown spatially here. Green lines indicate adjacent sampling months (November, March, April). Black lines indicate the Fv/Fm lows months. Note the Fv/Fm low point in both January and February occurs between 125 and 135km distance along the transect. This location is where the San Joaquin River enters Suisun Bay.

Seasonally, these sharp declines in Fv/Fm co-occurred with both low temperatures and high NH_4^+ concentrations in this region. However, full multiple regression ($\text{Fv/Fm} \sim \text{Temperature} + \text{Turbidity} + \text{NO}_3^- + \text{Chl } a + \text{Salinity} + \text{NH}_4^+$) for

the January cruise for the Suisun and LSR region ($R^2 = 0.96$) showed that Fv/Fm was strongly and significantly positively correlated with surface water temperature (coefficient 0.196 Fv/Fm $^{\circ}\text{C}^{-1}$ $p < 0.01$), significantly positively correlated with salinity (coefficient 0.002 Fv/Fm salinity unit $^{-1}$), significantly positively correlated with NH_4^+ (coefficient 0.004 Fv/Fm $\mu\text{mol NH}_4^+ \text{ L}^{-1}$, $p < 0.01$) and NO_3^- (coefficient 0.009 Fv/Fm $\mu\text{mol NO}_3 \text{ L}^{-1}$ $p < 0.01$), and negatively correlated with SPM (-0.0004 Fv/Fm $\mu\text{g SPM L}^{-1}$) and Chl *a* (-0.04 Fv/Fm $\mu\text{g Chl } a \text{ L}^{-1}$). Relative importance calculations show that 34% of the variance in Fv/Fm can be explained by changes in temperature (with colder water showing lower Fv/Fm), 21% to changes in Chl *a*, 17% to changes in NO_3^- , 16% to changes in salinity, 12% to changes in SPM, and 6% to changes in NH_4^+ . Similar results were found for the SUI/LSR Region in February, with an R^2 value of 0.94 and temperature explaining 24% of the variance (with turbidity playing a more important role (23% relative importance) in February). NH_4^+ and NO_3^- as predictors explained less than 3% combined of the variance in Fv/Fm in February. Results for all months are shown in Table 6-3.

The minimum Fv/Fm values in both months occurred at the confluence of the San Joaquin and Sacramento Rivers, with higher Fv/Fm values both farther upstream in the Sacramento River and farther downstream in SUI (Figure 6-11A). T-S plots reveal that the minimum Fv/Fm values are located precisely at the mixing point of water masses associated with the confluence (Figure 6-11B). Effectively, the low Fv/Fm in SUI in January 2015 and February 2015 is strongly driven by water temperatures associated with the influx of San Joaquin River water.

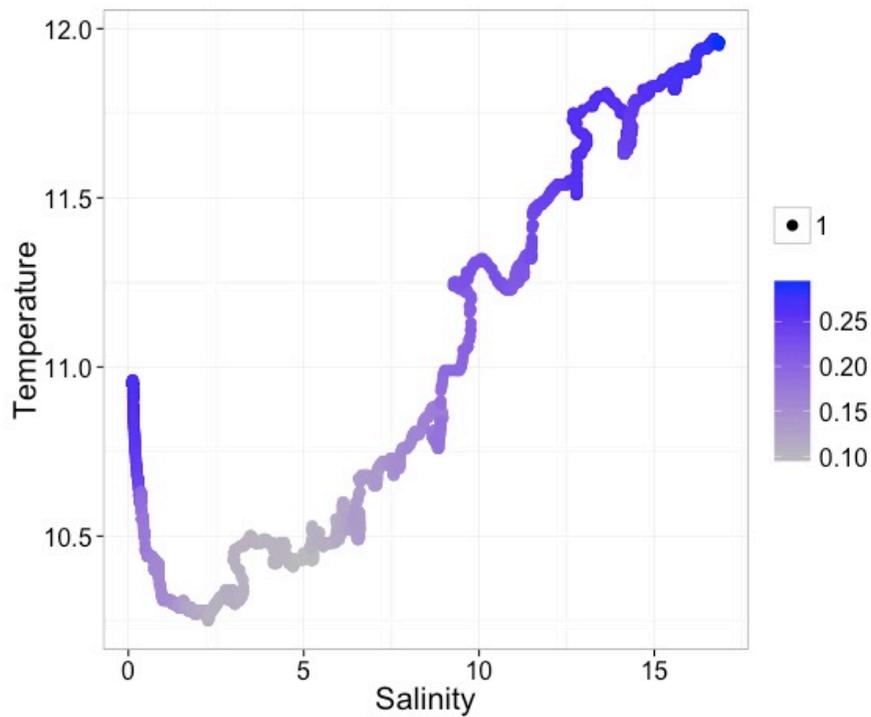
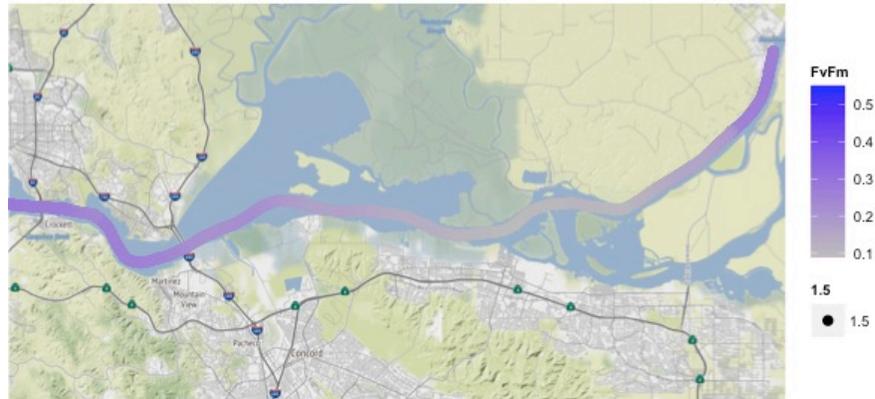


Figure 6-11. The primary locus of severely low F_v/F_m in January 2015 was situated at the confluence of the San Joaquin and Sacramento Rivers (A). This low F_v/F_m hotspot was also located at the point where water masses meet, as shown in a temperature-salinity plot from the same monthly cruise (B).

High-Frequency Events

High-frequency spatial sampling of DIN concentrations is not yet common practice in most water quality monitoring approaches. In addition to our specific examination of the variability of Fv/Fm throughout San Francisco Bay, and in particular the lower Fv/Fm values in SUI, we also probed our data to determine if high-frequency sampling revealed previously unknown locations of high-variability in DIN concentrations. In particular, given that the inputs of NH_4^+ to SF Bay are primarily from single point source POTWs, our data allowed us to determine if evidence of the plumes from these POTWs could be found within the cruise-track in the central ship-channel of the Bay.

We defined high-frequency events as locations, in time and space, of rapid change in under-way data as areas in which a given variable changed by >50% more than once across the average distance between discrete stations (~11 km) within the cruise track data of any given month.

Other than high NO_3^- concentrations in the extreme Lower South Bay, which are derived from tertiary treated effluent from the San Jose WWTP, changes in NO_3^- concentrations were gradual. A location of frequently high NH_4^+ concentration was identified in the western portion of SUI (38.0357°N, 122.1277°W, Figure 6-12) where concentrations spiked to 15-40 $\mu\text{mol L}^{-1}$ in near-surface waters over an area approximately 1.5-2 km wide. This feature had not previously been identified through discrete sampling approaches on regular water quality sampling cruises. Located near the mouth of the Contra Costa Sanitation District wastewater discharge from Pacheco

Creek, it was not present in all months, but was pronounced in September, October, and February. Similarly, a hotspot area of high NH_4^+ concentrations ($10\text{-}15 \mu\text{mol L}^{-1}$) was identified near the city of San Francisco, immediately north of the Oakland Bay Bridge in the months of September and October 2014.

Separately from the regularly high Chl *a* values observed locally in LSB throughout the spring months, several small-scale identifiable phytoplankton blooms were observed. From June through August 2014, a high turbidity ($>250 \text{ mg SPM L}^{-1}$) and high Chl *a* ($8\text{-}10 \mu\text{g Chl } a \text{ L}^{-1}$) patch was located in the central portion of South Bay. The area was approximately 3-4 km wide but was not associated with changes in nutrient concentrations or Fv/Fm. A small phytoplankton bloom was identified in the LSR in March 2015, peaking at $11.8 \mu\text{g Chl } a \text{ L}^{-1}$, and again in April 2015, peaking at $11.4 \mu\text{g Chl } a \text{ L}^{-1}$. A region of locally high Fv/Fm (>0.55) was also identified in March 2015 and April 2015 associated with the phytoplankton bloom in the LSR.

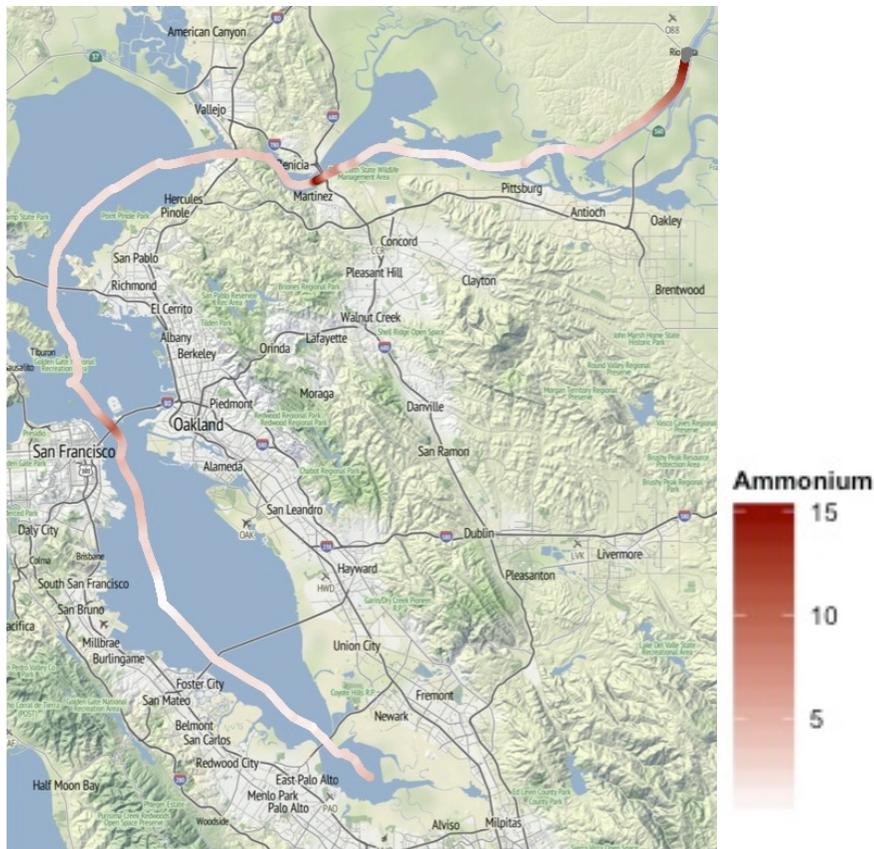


Figure 6-12 Hotspot NH_4^+ concentrations near San Francisco and in Western Suisun Bay.

DISCUSSION

For the first time, to our knowledge, simultaneous measurements of underway surface concentrations of NH_4^+ , NO_3^- , and surface phytoplankton Chl *a* and Fv/Fm were made in an estuarine system. Our measurements of NH_4^+ concentrations demonstrate that changes in riverine concentration of NH_4^+ delivered to the San Francisco Estuary from the Sacramento River drive the variability of NH_4^+ in SUI, rather than SUI NH_4^+ being driven by in situ or sediment N cycling processes, as suggested by Senn and Novick (2014). Thus, the seasonal concentrations of NH_4^+ in SUI are driven by changes in the concentration of NH_4^+ in river water entering SUI, which are correlated with riverine discharge (at least during this drought year). We

find greater NH_4^+ concentrations in winter than in summer (at least during this drought year). By simultaneously measuring NO_3^- and NH_4^+ , we found evidence of potential water column nitrification in only one region of SF Bay (the LSR) where NH_4^+ concentrations declined while NO_3^- concentrations increased.

Overall, our data reveal that phytoplankton photosynthetic yield is not uniformly driven by any one environmental variable. It is relatively insensitive to variations in nutrient availability (within this overall high-nutrient system). Instead, Fv/Fm is most sensitive to changes in salinity, temperature, Chl *a* and turbidity (as measured by SPM). While Chl *a* concentrations were predictably positively correlated with Fv/Fm, there was no consistent sign of the relationship between Fv/Fm and salinity, temperature or SPM across all seasons. Rather, what our data suggest is that the photosynthetic “health” of phytoplankton communities varies seasonally within different regions. And, overall, salinity and turbidity are the most important drivers of this variation in the summer, while temperature dominates in the winter.

The high concentrations of NH_4^+ in SUI have been suggested to be a cause of the low productivity of that region, by suppressing phytoplankton growth (Dugdale et al. 2007) or by changing phytoplankton community composition (Glibert 2010). Previous measurements of low Fv/Fm in the region have suggested that phytoplankton are physiologically damaged (Kudela 2014). Our continuous under-way measurements demonstrate that low Fv/Fm in SUI (the “Bad Suisun” phenomenon), while co-incident with seasonally high NH_4^+ concentrations in the LSR in the winter of 2014-2015, was not driven by NH_4^+ suppression by high concentrations of DIN. Rather, the low Fv/Fm in these months was strongly associated with San Joaquin

River water entering SUI, with higher Fv/Fm values both farther upstream in the LSR (with higher NH₄⁺ concentrations) and farther downstream in SUI (with lower NH₄⁺ concentrations). Multiple-regression analysis revealed that low Fv/Fm is strongly controlled by water temperature. It has been also been suggested that seasonal blooms occur when low-flow conditions lower NH₄⁺ concentrations below a threshold (Dugdale et al. 2007). However, we observed bloom Chl *a* values and high Fv/Fm values in the LSR in March and April 2015, co-incident with NH₄⁺ concentrations > 4μM.

So what is causing the “Bad Suisun” phenomenon? First, while Fv/Fm is significantly lower year-round in SUI relative to all other embayments, these differences were largest during in the winter months, and in particular, there was a strong and significant correlation with Fv/Fm and temperature, with lower temperatures correlated with lower Fv/Fm. Across the entire SF Bay, temperature plays a much larger role in Fv/Fm variability in the winter months.

However, temperature alone cannot be the cause of low Fv/Fm because Fv/Fm is significantly lower in SUI than in other regions of the Bay throughout the year. One possibility is that the change in Fv/Fm observed in the region is representative of a shift in phytoplankton community assemblage in SUI relative to surrounding waters. However, previous work has demonstrated that the SUI assemblage is largely dominated by diatom species and there is significant overlap in community composition between this region and neighboring regions (Esparza et al. 2014). The cause of low Fv/Fm to the region appears, at least in this year and in the winter months where it was most pronounced, to originate from the San Joaquin River.

The Advantages of High Frequency Data

Taken as a whole, our high-frequency underway sampling of both DIN species revealed only a small handful of phenomena that had been “missed” by regular, long-term discrete surface water samples, such as an NH_4^+ hotspot adjacent to a wastewater treatment plant discharge site. In large part, high-frequency sampling reproduced the spatial and seasonal patterns identified through widely-spaced discrete sampling. When discrete data are collected at widely spaced stations, the volume of data generated is relatively small, and the ability to have confidence in the drivers of observed variability is minimal, both conceptually and statistically (Jassby et al. 1997). However, the conversion of DIN measurements to an under-way full-coverage dataset makes additional analyses possible because the data coverage is comprehensive across many spatial scales, allowing for the use of correlational and regression analyses with large volumes of data.

Such insights into N dynamics are useful not only to our ecological understanding the biogeochemistry and ecology of estuarine and coastal systems, but also to water quality managers and other environmental decision-makers in the United States who seek to use information about nutrient states to assess the impairment of water bodies under Section 303d of the Clean Water Act and within Total Maximum Daily Load designation processes. There has been increasing interest among water quality managers in 1) tracking and attributing anthropogenic DIN in human-impacted water bodies to point and non-point sources (Fowler et al. 2013), 2) understanding the fate of DIN within ecosystems, and 3) developing tools to assess the conditions under

which DIN has significant ecological consequences. In 1998, the US EPA announced a National Strategy for the Development of Regional Nutrient Criteria. Water quality scientists and managers in the San Francisco Bay area have since been working to develop Numerical Nutrient Endpoint (NNE) criteria that would link specific concentrations of NH_4^+ and NO_3^- to observed negative ecological consequences. To date, the result of this process has been the development of a Nutrient Management Strategy, which included the new 2014 National Pollution Discharge Elimination System (NPDES) permits for wastewater dischargers into San Francisco Bay. The governance structure, technical details and implementation of the strategy are undergoing further development (SF Bay Regional Water Quality Control Board, 2014).

CONCLUSIONS

Here, we demonstrate the utility of a high-spatial frequency ship-deployable NH_4^+ analyzer that accurately measures surface NH_4^+ concentrations underway. When coupled with an optical NO_3^- sensor and a variable fluorescence fluorometer, coupled measurements of DIN concentrations and Fv/Fm can now be a constituent part of an under-way water quality monitoring data-set. To our knowledge, this is the first study that has ever measured all three of these environmental parameters (NH_4^+ , NO_3^- and Fv/Fm) as continuous surface variables in an estuary.

In using this approach, we are able to demonstrate that the “Bad Suisun” phenomenon of low photosynthetic yield in northern SFBD is not driven by nutrient loading, but is strongly seasonal and related to changes in temperature, salinity, and

turbidity. We also find that the overall physiological state of phytoplankton throughout SFBD is largely driven by changes in water masses, environmental variables, and by increases in chlorophyll concentration, rather than by changes in nutrient availability.

Comprehensive water quality sampling can help inform nutrient management strategies like the one in San Francisco Bay by highlighting likely sources of nutrient loading, providing data to accurately model and predict future N dynamics, and by examining the spatial relationship of nutrient concentrations to phytoplankton physiological state or other ecological variables. The regular use of such approaches, especially when coupled with moored sensors to capture temporal variability at single locations, will help fill in the gaps to paint a comprehensive vision of the dynamics of anthropogenic perturbation of the N cycle in this human-impacted estuary.

Dissertation Conclusions

This dissertation has addressed six separate research questions, linked through three thematic cases, each of which speaks to a dimension of sustainability science needed to address human alteration of the carbon and nitrogen cycles within the social-ecological systems framework. The individual conclusions of each chapter are directly relevant to the scholars working within the sub-disciplines represented by the three themes, and to the practitioners seeking to address these problems at local, regional, and national scales. Part I showed that ecosystem-specific responses of the carbon cycle to anthropogenic global changes determine the sign and magnitude of potential carbon cycle feedbacks (if any) to anthropogenic climate change from ecosystem carbon sources and sinks. In particular, I showed that strong seasonal carbon sinks may not sequester carbon in the long-term on continental shelves, and that increases in CO₂ emissions from soils in response to disturbance may not represent an increase in a GHG source, but rather an acceleration of the rate of carbon cycling. Part II demonstrated that new approaches to managing blue carbon ecosystem services require careful attention to the contexts and modalities of the uptake of this framework. In particular, I showed that there is resistance to the ecosystem services framework among local, place-based conservation organizations working on conservation practice that is motivated by a concern that the ecosystem services delivered by carbon sequestration in ecosystems are globally diffuse and not delivered locally. I also showed that current regulatory practice treats blue carbon as an implicit offset to mitigate other emissions. These results together illustrate the ways in which stewardship of ecosystems in the Anthropocene is as dependent on social and

institutional receptivity to science as it is on the state of scientific knowledge. Part III engaged with the sustainability science paradigm in a different way: directing scientific inquiry toward a management-relevant set of scientific questions about the impacts of a particular anthropogenic perturbation of the nitrogen cycle in a coastal ecosystem. Together, the results of this work provide experimental and observational evidence, contrary to previously published findings, that wastewater effluent is not driving the low phytoplankton productivity in the San Francisco Bay estuary, with implications for N cycle management under the Clean Water Act.

Taken as a whole, this dissertation as a body of work does not seek to produce categorical conclusions about the behavior and dynamics of social-ecological systems across these examples. Rather, through each of these studies, it aims to contribute to our understanding of sustainability science by producing new, management-relevant scientific knowledge about anthropogenic global change, developing our understanding of what can make adaptive environmental management work in the political, ecological and social contexts of rapid global change, and guiding the evolution of new institutional approaches to environmental management. I hope that I have been successful in that endeavor.

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