UNIVERSITY OF CALIFORNIA, MERCED

Nitrogen cycling in a changing world: Response of a California grassland to multiple global change factors

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

In

Environmental Systems

by

Chelsea J. Carey

Committee in charge:

Stephen C. Hart, Chair
Michael Beman
Valerie T. Eviner
Lara Kueppers

The dissertation of Chelsea Carey is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

_____

Michael Beman

_____

Valerie T. Eviner

_____

Lara Kueppers

_____

Stephen C. Hart, Chair

University of California, Merced
2014

Table of Contents

List of Tables

List of Figures

Acknowledgements

True teachers use themselves as bridges over which they invite their students to cross; then, having facilitated their crossing, joyfully collapse, encouraging them to create bridges of their own. – Nikos Kazantzakis

The completion of this dissertation would not have been possible without the support and guidance of many people over the past five years and beyond. I have been incredibly fortunate to have many amazing teachers inside the classroom and out. In the following pages, I will give thanks to a few of the many who have impacted my education and helped me get to where I am today.

To begin, I'd like to extend my fullest gratitude to my major advisor, Stephen C. Hart, for allowing me to pursue my research interests, for supporting me every step of the way, and for being a continual source of inspiration. Steve's support of my ideas – which weren't always in line with his current research foci – is a testament to his interest in all things ecology, amazing breadth of knowledge, and willingness to support his students. Steve has an unprecedented passion for science, evidenced by his eagerness to conduct field and laboratory work (not every advisor would help sieve soil or run hundreds of KCl extractions!), which is infectious and inspiring. As my major advisor, teacher, and boss, Steve has been a constant role model in my life for the past five years. I will forever be grateful to him for taking the time to be a wonderful mentor and for nurturing my growth as a scientist.

I'd also like to thank my other committee members, Valerie Eviner, Lara Kueppers, and Mike Beman, for their instruction and timely feedback. Valerie graciously let me join an existing project that she and Drs. Carolyn Malmstrom and Kevin Rice had developed, which ended up being where I conducted all of my fieldwork. Without her willingness to share her experimental site, knowledge, and time, my project surely would not have been what it is today. Mike was instrumental in developing the molecular portion of my Ph.D. work, and I cannot thank him enough for allowing me to use his laboratory, for taking time to work on my NSF fellowship so that it was award-worthy, and for helping make sense of my sometimes not-so-straightforward results. Finally, a big thank you to Lara for always having an open door and for providing great insights into the writing process throughout the years. Together with my major advisor, I could not have asked for a better group of mentors.

My research would not have been completed without the help of those in the Hart Lab. I have had the pleasure of working with a group of undergraduate research assistants that could be described as nothing less than rockstars. Abby Dziegiel, Grant Ivison-Lane, Ariel Hickman, and Nick Marlowe helped me get through many long field days, hours of soil sieving, and hundreds of soil extractions. Also, thank you to my lab mates, Emma McCorkle and Erin Stacy, for providing years of friendship, and for helping me in the

field and laboratory. I would not have made it through those hot summer days of sampling and long days by the Lachat without you. Special thanks to Joey Blankinship for showing me how to run the gas chromatograph, teaching me many lab assays, and always providing quick revisions of my manuscripts.

I'd also like to extend my gratitude to Jill Baty for working with me at the experimental site in Davis and for donating soil samples to my project in the spring of 2011, and to Joanne Heraty for providing updates and details on site management over the years. Recognition should also be extended to Liying Zhao for her help running and troubleshooting instruments in the Sierra Nevada Research Institute Environmental Analytical Laboratory.

A special thanks to my undergraduate advisor, Liam Heneghan, and lab coordinator, Lauren Umek, for introducing me to the wonderful world of DIRT. By allowing me to explore the ecology of Chicago and conduct research for the first time, Liam and Lauren had an immense influence on the trajectory of my life. Surely I would not be here today if it weren't for them. I would also like to send a "Miniracer shout out" to my previous labmates, Vanessa Coffman and Ryan Wietholter, and to the rest of the Environmental Science Department at DePaul University – Judy Bramble, Jim Montgomery, and Maggie Workman. You all made science and life at DePaul magical!

To my California family who helped me retain some semblance of sanity over the past five years, I am forever grateful. Special thanks to Kristynn Sullivan and Alyssa Carrell for being there through the highs and lows (typically with wine), for helping take care of my dog when I couldn't, and for humoring my questions on statistics and sequencing. To Nick Valdez, my best friend and partner, thank you for always reminding me to take a break, for believing in me even when I didn't, and for providing unwavering support during this journey. Finally, a big thank you to all the others who have provided camaraderie over the years and who have made California my home away from home, including (but not limited to!), Katie Amrine, Chelsea Arnold, Mike Bell, Rebecca Lever, Derek Merrill, Ivy Price, Steven Scott, Tracy White, The Valdez family, and my two dogs, Dia and LG.

Most of all, I'd like to thank my family – Mom, Dad, Brook, Brandon, Owen, Jack, and Angie – for being my everything. You are the best, most supportive family a girl could ask for and I would not have made it a day in this world if it weren't for your unconditional love.

And of course, last but not least, I must thank the funding sources that helped pay for my dissertation research over the past five years. UC Merced's Graduate Summer Fellowships funded many summers of research, and the molecular portion of my work was paid for by the National Science Foundation's Doctoral Dissertation Improvement Grant (DEB-1311388).

Curriculum Vitae

EDUCATION

Doctor of Philosophy in Environmental Systems 2009-2014
University of California, Merced *Merced, California*

Bachelor of Science in Biology and Environmental Science 2004-2008
DePaul University *Chicago, Illinois*

JOURNAL PUBLICATIONS

Carey, C.J., Blankinship, J., Eviner, V.T., and S.C. Hart. *In Prep.* Exotic plant invasion slows nitrogen cycling in a California grassland.

Carey, C.J., Beman, M., Blankinship, J., Eviner, V.T., and S.C. Hart. *In Prep.* Nitrogen fertilization and defoliation influence soil nitrogen cycling but not the abundance of ammonia oxidizing microorganisms in an invaded grassland community.

Carey, C.J., Beman, M., Eviner, V.T., and S.C. Hart. *In Prep.* Microbial community composition is insensitive to multiple global changes.

Carey, C.J. 2008. The impact of corn (*Zea mays*) on restoration efforts in buckthorn (*Rhamnus cathartica*) invaded soils. DePaul University Liberal Arts and Sciences Student Research Journal 1: 18-20.

CONFERENCE ABSTRACTS

Linking global-change induced shifts in soil nitrogen cycling with the abundance of key microorganisms. American Geophysical Union Meeting. December 13, 2013.

Response of soil nitrogen cycling to the interaction of invasive plants, simulated grazing, and nitrogen additions. California Invasive Plant Council Symposium. October 3, 2013.

Interactive effects of multiple global change factors on soil nitrogen transformations in a California grassland. Ecological Society of America Annual Meeting. August 9, 2012.

Alteration of nitrogen cycling processes by exotic annuals in a California grassland. California Invasive Plant Council Symposium. October 5, 2011.

The Impact of Corn (*Zea mays)* on Restoration Efforts in Buckthorn (*Rhamnus cathartica*) Invaded Soils. Midwest Ecology and Evolution Conference. March 9, 2008.

AWARDS

2013 National Science Foundation Dissertation Improvement Grant
2013 UCM Environmental Systems Program Graduate Student of the Year Award
2013 UCM Environmental Systems Graduate Fellowship
2012 Ecological Society of America Soil Ecology Student Travel Award
2011 UCM Environmental Systems Graduate Bobcat Fellowship
2010 UCM Graduate Student Fellowship
2008 DePaul University Environmental Science Scholarship

Abstract of the Dissertation

Nitrogen cycling in a changing world: Response of a California grassland to multiple global change factors

by

Chelsea J. Carey

Doctor of Philosophy, Environmental Systems Program
University of California, Merced, 2014
Dr. Stephen C. Hart, Chair

The cycling of nutrients, such as nitrogen (N), is arguably one of the most critical ecosystem services provided by soil. Nitrogen is the limiting nutrient for plant growth in many terrestrial ecosystems and can consequently regulate net primary production, plant diversity, and community composition. Transformations of available N, which are catalyzed by soil microorganisms, can also affect air and water quality, with possible implications for climate change and human health. In an era of global environmental change, it is paramount to gain a mechanistic understanding of how soil N is affected by anthropogenically derived perturbations such as exotic plant invasion and elevated nutrient deposition. Using a multifactor global change experiment, I assessed how three principal global change factors – exotic plant invasion, N deposition (simulated by N fertilization), and aboveground vegetation removal (to simulate cattle grazing or mowing) – affected soil N cycling, namely NH_4^+ and NO_3^- availability and potential rates of nitrification and denitrification, in a California grassland. In order to increase understanding of how soil microbial communities regulate changes in N cycling, I concurrently measured broad-scale community structure of bacteria and archaea and the abundances of ammonia-oxidizing bacteria and archaea. I found that two invasive plants, *Aegilops triuncialis* and *Elymus caput-medusae*, reduced soil N availability and nitrification and denitrification potentials compared to perennial-dominated native communities but not naturalized exotic communities. Aboveground vegetation removal, which is often used as a tool to manage invasive plant populations (through cattle grazing or mowing), tended to exacerbate the effects of invasion by further reducing nitrification potential and soil NO_3^- availability. Fertilization with NH_4NO_3 consistently increased nitrification potential and soil NO_3^- availability, yet NH_4^+ remained unaffected and

denitrification potential was reduced. When combined, defoliation and N fertilization always produced additive effects. Finally, despite the sometimes dramatic shifts in N availability and potential rates that were observed, microbial community composition remained unaffected by changes in plant composition, N fertilization and defoliation. Overall, these findings provide evidence that N cycling is uniquely affected by each individual global change factor, and that the interactive effects of N fertilization and defoliation can be predicted based on combining single factor studies. These results also suggest that microbial communities composition is insensitive to global change in this system, and that microbial activity – as measured by rates of N cycling – is decoupled from community composition.

1 Introduction

Soils are complex systems comprised of pore space, minerals, and organic matter. A diverse group of macro- and micro-organisms reside in soil, where nutrients are transformed, gases are produced and consumed, and water is transported. In this way, soil supports valuable ecosystem services - e.g., plant growth, water and air quality, and habitat for soil-dwelling organisms (Daily *et al.*, 1997). The cycling of nutrients, such as nitrogen (N), is arguably one of the most critical functions affecting the provision of ecosystem services by soil. Nitrogen is the limiting nutrient for plant growth in most terrestrial systems (Vitousek & Howarth, 1991); as such, soil N availability regulates net primary production (NPP; LeBauer & Treseder, 2008), plant diversity (Bobbink et al., 2010), and community composition (Tilman, 1987; Huenneke *et al.*, 1990; McClean *et al.*, 2011). Moreover, transformations of available N via nitrification (NH_4^+ to NO_3^-) and denitrification (NO_3^- to N_2) produce nitrous oxide (N_2O; Bateman & Baggs, 2005; Parton et al., 2012), a critical greenhouse gas, and nitric oxide (NO; Bollmann & Conrad, 1998), which is quickly transformed into the air pollutant nitrogen dioxide (NO_2). Water quality is also affected by soil N cycling; nitrate, and to a lesser extent NH_4^+, can leach into groundwater (MacDonald *et al.*, 2002; Rowe *et al.*, 2006; Jahangir *et al.*, 2012), create eutrophication of adjacent aquatic ecosystems (Smith *et al.*, 1999), and lead to unsafe drinking water conditions (Ward *et al.*, 2005). The regulation of N cycling thus has potentially large consequences for maintenance of soil-derived ecosystem services.

Soil microorganisms are responsible for catalyzing key processes in the N cycle (Figure 1-1). For instance, heterotrophic bacteria and fungi excrete extracellular enzymes to access C contained in organic matter (Sinsabaugh, 1994); as a "byproduct" of this search for energy, organically bound N is released into the soil solution as a microbial and plant available form (NH_4^+; McGill & Cole, 1981; Zumft, 1997; Schimel & Bennett, 2004). A small group of microorganisms (ammonia oxidizing bacteria and archaea) can then utilize the NH_4^+ that is released as a source of electrons to produce adenosine triphosphate (ATP; Hofman & Lees, 1953; Stahl & la Torre, 2012), a key energy molecule. The byproduct, NO_2^-, is utilized in the same way by nitrite oxidizers (Lees & Simpson, 1957), and together, these two steps make up nitrification. A phylogenetically broader group of microorganisms (Shoun *et al.*, 1992; Philippot, 2002), including bacteria, archaea, and fungi, utilize NO_3^- as a terminal electron acceptor during anaerobic respiration, thereby reducing NO_3^- to NO, N_2O and finally N_2 (i.e., denitrification), which is released into the

atmosphere. Clearly, these processes are inextricably linked to the microorganisms that catalyze them, suggesting that the composition, relative abundances, and richness (together = 'structure') of the microbial community should be of importance for soil N cycling and availability. Results from an increasing amount of studies, however, demonstrate that the relationship between N cycling and microbial community structure is not straightforward (Swift *et al.*, 1998; Cavigelli & Robertson, 2000; Griffiths *et al.*, 2000; Setälä & McLean, 2004; Salles, 2012; Weedon *et al.*, 2012) even for specialized processes such as nitrification (Prosser & Nicol, 2008; Hallin *et al.*, 2009; Di *et al.*, 2009; Yao *et al.*, 2011), highlighting the need for continued research.

Understanding how microbial community structure regulates soil N cycling is becoming even more crucial with accelerated global change. Microorganisms differ in their sensitivity to biotic (e.g., changes in plant composition) and abiotic (e.g., temperature and precipitation) variables; as such, global change could favor certain groups of microorganisms over others (Fierer *et al.*, 2007; Verhamme *et al.*, 2011; Ramirez *et al.*, 2012), leading to shifts in microbial composition, diversity, and subsequent N cycling. To understand if this is the case, numerous studies have manipulated one or more global change factor(s) and monitored the response of either soil N cycling (e.g., Barnard *et al.*, 2006; Liao *et al.*, 2008; Niboyet *et al.*, 2010; Dijkstra *et al.*, 2010; Brown *et al.*, 2011; Ochoa-Hueso *et al.*, 2013) or the microbial community (e.g., Horz *et al.*, 2004; Zhang *et al.*, 2005; Batten *et al.*, 2006; Treseder, 2008; Castro *et al.*, 2010; He *et al.*, 2010; Shen *et al.*, 2011; Ramirez *et al.*, 2012; Gutknecht *et al.*, 2012); however, relatively few have monitored soil N cycling and microbial community composition from the same treatments, especially in multifactor global change experiments. This is a large research need, given that multiple global change factors often simultaneously influence ecosystems and likely lead to unanticipated interactive effects.

The overall aim of my dissertation was to quantify the single and combined effects of three principle global change factors affecting Mediterranean grasslands today: exotic plant invasion, nitrogen (N) deposition, and aboveground vegetation removal (one component of cattle grazing). In California especially, these factors are of particular importance, given their high occurrences on the landscape. Indeed, California is home to over 200 invasive plants (Cal-IPC) and 5 million cattle (USDA NASS 2012), and experiences some of the highest N deposition rates in North America ($\geq$ 90 kg N ha^{-1} y^{-1} in some areas; Fenn et al., 2010). Understanding how factors, such as these, interact will help provide a more realistic approximation of ecosystem dynamics in the future. This understanding may increase management success by helping land managers identify scenarios where the mitigation of one global change driver may require the concurrent mitigation of another global change driver acting simultaneously.

In order to increase understanding of how soil microbial communities drive ecosystem response to global change, I measured the response of both N cycling processes and soil microbial community structure to exotic plant invasion, N deposition, and aboveground vegetation removal. Additionally, because Mediterranean-type ecosystems experience

strong seasonality in soil temperature, soil water content, and plant phenology, I evaluated if and how the relative effects of these global change factors varied throughout the year. All data was collected from a well-replicated multifactor global change field experiment located in Davis, CA.

1.1 Organization of the Dissertation

The research from this dissertation is divided into three self-contained chapters (2, 3, and 4), which are written in manuscript format. In Chapter two, "Exotic plant invasion slows nitrogen cycling in a California grassland," I investigated how soil N cycling differed among native grassland communities, naturalized communities (i.e., communities of exotic species that are now so ubiquitous that they are considered part of the native flora), and communities comprised of two aggressive invasive species, *Aegilops triuncialis* and *Elymus caput-medusae*. This chapter will be submitted to the journal, Ecology. In the third chapter, "Nitrogen fertilization and defoliation influence soil nitrogen cycling but not the abundance of ammonia oxidizing microorganisms in an invaded grassland community," I first compared N cycling of native communities to invaded mixtures (native, naturalized, and invasive species), then determined how N fertilization, aboveground vegetation removal, and their interaction affected soil N cycling and the abundances of key microorganisms (ammonia oxidizing bacteria and archaea) in invaded grasslands. This chapter will be submitted to Global Change Biology. In the fourth chapter, "Microbial community composition is insensitive to multiple global changes in experimental grasslands," I used next-generation sequencing techniques to determine if bacterial and archaeal community structures were affected by exotic plant invasion, N fertilization, aboveground vegetation removal, or the interaction of N fertilization and aboveground vegetation removal. This chapter will be submitted to the ISME Journal. All chapters were written using the pronoun "we", which refers to co-authors of the manuscript and myself.

1.2 References

Barnard R, Le Roux X, Hungate BA, Cleland EE, Blankinship JC, Barthes L, Leadley PW (2006) Several components of global change alter nitrifying and denitrifying activities in an annual grassland. *Functional Ecology*, **20**, 557–564.

Bateman EJ, Baggs EM (2005) Contributions of nitrification and denitrification to N2O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils*, **41**, 379–388.

Batten KM, Scow KM, Davies KF, Harrison SP (2006) Two Invasive Plants Alter Soil Microbial Community Composition in Serpentine Grasslands. *Biological Invasions*, **8**, 217–230.

Bobbink R, Hicks K, Galloway J, et al. (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications*, **20**, 30–59.

Bollmann A, Conrad R (1998) Influence of O2 availability on NO and N2O release by nitrification and denitrification in soils. *Global Change Biology*, **4**, 387–396.

Brown JR, Blankinship JC, Niboyet A, et al. (2011) Effects of multiple global change treatments on soil N2O fluxes. *Biogeochemistry*, **109**, 85–100.

Castro HF, Classen AT, Austin EE, Norby RJ, Schadt CW (2010) Soil Microbial Community Responses to Multiple Experimental Climate Change Drivers. *Applied and Environmental Microbiology*, **76**, 999–1007.

Cavigelli MA, Robertson GP (2000) The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology*, **81**, 1402–1414.

Daily GC, Matson PA, Vitousek PM (1997) Ecosystem services supplied by soil. In: *Nature's Services: Societal Dependence on Natural Ecosystems* Daily GC pp113–133. Washington, DC, Island Press.

Di HJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S, He JZ (2009) Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nature Geoscience*, **2**, 621–624.

Dijkstra FA, Blumenthal D, Morgan JA, Pendall E, Carrillo Y, Follett RF (2010) Contrasting effects of elevated CO2 and warming on nitrogen cycling in a semiarid grassland. *New Phytologist*, **187**, 426–437.

Fenn ME, Allen EB, Weiss SB, et al. (2010) Journal of Environmental Management. *Journal of Environmental Management*, **91**, 2404–2423.

Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. *Ecology*, **88**, 1354–1364.

Griffiths BS, Ritz K, Bardgett RD, et al. (2000) Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity–ecosystem function relationship. *Oikos*, **90**, 279–294.

Gutknecht JLM, Field CB, Balser TC (2012) Microbial communities and their responses to simulated global change fluctuate greatly over multiple years. *Global Change Biology*, **18**, 2256–2269.

Hallin S, Jones CM, Schloter M, Philippot L (2009) Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. *The ISME Journal,* **3**, 597–605.

He Z, Xu M, Deng Y, et al. (2010) Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO2. *Ecology Letters*, **13**, 564–575.

Hofman T, Lees H (1953) The biochemistry of the nitrifying organisms. 4. The respiration and intermediary metabolism of Nitrosomonas. *Biochemical Journal*, **54**, 579.

Horz H-P, Barbrook A, Field CB, Bohannan BJM (2004) Ammonia-oxidizing bacteria respond to multifactorial global change. *Proceedings of the National Academy of Sciences*, **101**, 15136–15141.

Huenneke LF, Hamburg SP, Koide R, Mooney HA, Vitousek PM (1990) Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. *Ecology*, **71**, 478–491.

Jahangir M, Johnston P, Khalil MI, Hennessy D, Humphreys J, Fenton O, Richards KG (2012) Groundwater: A pathway for terrestrial C and N losses and indirect greenhouse gas emissions. *Agriculture, Ecosystems & Environment*, **159**, 40–48.

LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, **89**, 371–379.

Lees H, Simpson JR (1957) The biochemistry of the nitrifying organisms. 5. Nitrite oxidation by Nitrobacter. *Biochemical Journal*, **65**, 297–305.

Liao C, Peng R, Luo Y, et al. (2008) Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. *New Phytologist*, **177**, 706–714.

MacDonald JA, Dise NB, Matzner E, Armbruster M, Gundersen P, Forsius M (2002) Nitrogen input together with ecosystem nitrogen enrichment predict nitrate leaching from European forests. *Global Change Biology*, **8**, 1028–1033.

McClean CJ, Berg LJL, Ashmore MR, Preston CD (2011) Atmospheric nitrogen deposition explains patterns of plant species loss. *Global Change Biology*, **17**, 2882–2892.

McGill WB, Cole CV (1981) Comparative aspects of cycling of organic C, N, S and P through soil organic matter. *Geoderma*, **26**, 267–286.

Niboyet A, Barthes L, Hungate BA, et al. (2010) Responses of soil nitrogen cycling to the interactive effects of elevated CO2 and inorganic N supply. *Plant and Soil*, **327**, 35–47.

Ochoa-Hueso R, Maestre FT, de los Ríos A, et al. (2013) Environmental Pollution. *Environmental Pollution*, **179**, 185–193.

Parton WJ, Mosier AR, Ojima DS, Valentine DW, Schimel DS, Weier K, Kulmala AE (2012) Generalized model for N2 and N2O production from nitrification and denitrification. *Global Biogeochemical Cycles*, **10**, 401–412.

Philippot L (2002) Denitrifying genes in bacterial and Archaeal genomes. *Biochimica et biophysica acta*, **1577**, 355–376.

Prosser JI, Nicol GW (2008) Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. *Environmental Microbiology*, **10**, 2931–2941.

Ramirez KS, Craine JM, Fierer N (2012) Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, **18**, 1918–1927.

Rowe EC, Evans CD, Emmett BA, Reynolds B, Helliwell RC, Coull MC, Curtis CJ (2006) Vegetation Type Affects the Relationship Between Soil Carbon to Nitrogen Ratio and Nitrogen Leaching. *Water, Air, & Soil Pollution*, **177**, 335–347.

Salles JF (2012) Relating phylogenetic and functional diversity among denitrifiers and quantifying their capacity to predict community functioning. *Frontiers in Microbiology,* **3**, 1–15.

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. *Ecology*, **85**, 591–602.

Setälä H, McLean MA (2004) Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. *Oecologia*, **139**, 98–107.

Shen X-Y, Zhang L-M, Shen J-P, Li L-H, Yuan C-L, He J-Z (2011) Nitrogen loading levels affect abundance and composition of soil ammonia oxidizing prokaryotes in semiarid temperate grassland. *Journal of Soils and Sediments*, **11**, 1243–1252.

Shoun H, Kim DH, Uchiyama H, Sugiyama J (1992) Denitrification by fungi. *FEMS Microbiology Letters*, **94**, 277–281.

Sinsabaugh RS (1994) Enzymic analysis of microbial pattern and process. *Biology and Fertility of Soils*, **17**, 69–74.

Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution*, **100**, 179–196.

Stahl DA, la Torre de JR (2012) Physiology and Diversity of Ammonia-Oxidizing Archaea. *Annual Review of Microbiology*, **66**, 83–101.

Swift MJ, Andren O, Brussaard L, et al. (1998) Global change, soil biodiversity, and nitrogen cycling in terrestrial ecosystems: three case studies. *Global Change Biology*, **4**, 729–743.

Tilman D (1987) Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. *Ecological Monographs*, **57**, 189–214.

Treseder KK (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters*, **11**, 1111–1120.

Verhamme DT, Prosser JI, Nicol GW (2011) Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. *The ISME Journal*, **5**, 1067–1071.

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry*, **13**, 87–115.

Ward MH, deKok TM, Levallois P, Brender J, Gulis G, Nolan BT, VanDerslice J (2005) Workgroup report: Drinking-water nitrate and health—recent findings and research needs. *Environmental Health Perspectives*, **113**, 1607–1614.

Weedon JT, Kowalchuk GA, Aerts R, et al. (2012) Summer warming accelerates sub-arctic peatland nitrogen cycling without changing enzyme pools or microbial community structure. *Global Change Biology*, **18**, 138–150.

Yao H, Gao Y, Nicol GW, et al. (2011) Links between Ammonia Oxidizer Community Structure, Abundance, and Nitrification Potential in Acidic Soils. *Applied and Environmental Microbiology*, **77**, 4618–4625.

Zhang W, Parker KM, Luo Y, Wan S, Wallace LL, Hu S (2005) Soil microbial responses to experimental warming and clipping in a tallgrass prairie. *Global Change Biology*, **11**, 266–277.

Zumft WG (1997) Cell biology and molecular basis of denitrification. *Microbiology and Molecular Biology Reviews*, **61**, 533–596.

1.3 Figures

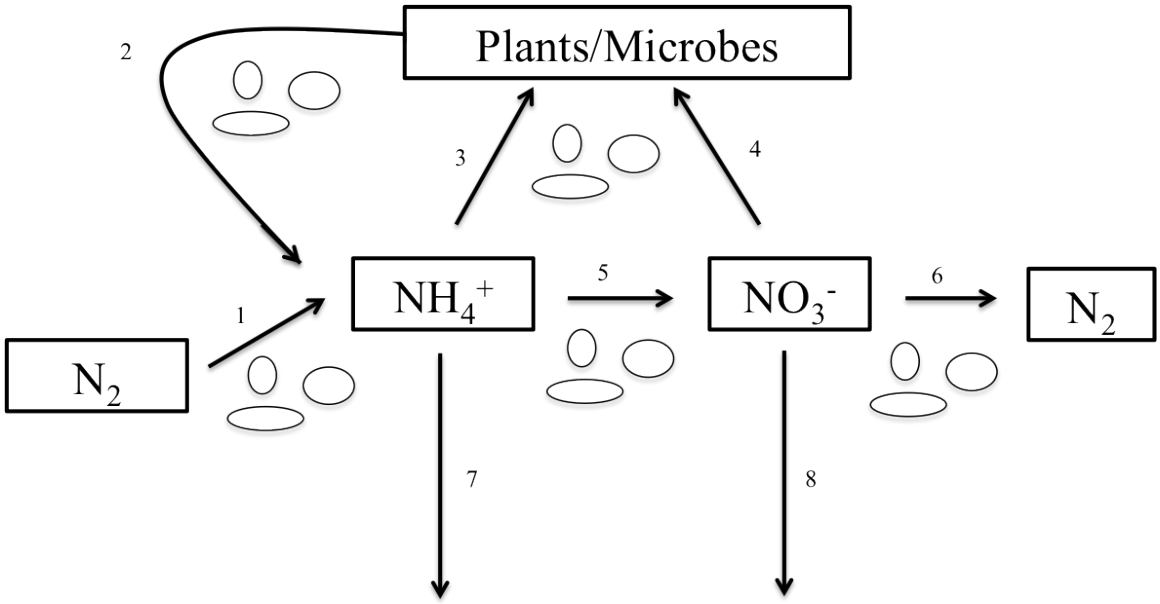

Figure 1-1 Simplified diagram of the nitrogen (N) cycle. 1) N fixation, 2) N mineralization/ammonification, 3) microbial immobilization or plant uptake of NH_4^+, 4) microbial immobilization or plant uptake of NO_3^-, 5) nitrification, 6) denitrification, 7) leaching of NH_4^+, 8) leaching of NO_3^-. Steps 1-6 are mediated by microorganisms, which are denoted by the small open circles. Plant uptake of N (steps 3 and 4) can be, but is not necessarily, facilitated by microorganisms.

2 Exotic plant invasion slows nitrogen cycling in a California grassland

2.1 Abstract

Invasive species constitute a significant component of global environmental change. In California grasslands, annual exotic grasses have been replacing native species since European colonization, many of which are now so ubiquitous that they are considered part of the regional flora ('naturalized'). New invasive plants, such as *Aegilops triuncialis* (Barb goatgrass) and *Elymus caput-medusae* (Medusahead), continue to spread throughout the state today. It is critical to understand how exotic plant invasion affects nitrogen (N) cycling, given that changes in N availability can have consequences for vegetation dynamics, restoration efforts, and air and water quality. This study compared soil N cycling and microbial biomass of experimental grasslands comprised of (1) invasives *A. triuncialis* and *E. caput-medusae*, (2) exotic annual species that are now naturalized, and (3) native annual and perennial species. We found that *A. triuncialis* and *E. caput-medusae* significantly reduced N availability and nitrification and denitrification potentials compared to native communities. These differences were associated with lower microbial biomass and volumetric soil moisture in surface soil. In contrast, soil N cycling was similar between invaded and naturalized communities, suggesting that invasion of naturalized grasslands by *A. triuncialis* and *E. caput-medusae* may not induce changes in soil N cycling.

2.2 Introduction

Invasive species occupy many ecosystems worldwide and are considered an important component of global environmental change (Vitousek *et al.*, 1997; Wilcove *et al.*, 1998; Sala *et al.*, 2000; Tylianakis *et al.*, 2008). With over 2,100 established invasive vascular plants in the United States (Vitousek *et al.*, 1997), an annual management cost of $27 billion (Pimentel *et al.*, 2005), and the potential for significant increases in invasive species establishment (Sala *et al.*, 2000), many studies have focused on understanding the mechanisms by which invasive species establish, persist, and spread (e.g., Mack *et al.*, 2000; Davidson *et al.*, 2011; Bradley *et al.*, 2012; Drenovsky *et al.*, 2012). Additionally, a growing number of studies have sought to understand the effects of invasive plants on

key ecosystem processes, such as nutrient cycling and net primary production (Vitousek *et al.*, 1987; Ehrenfeld, 2003; Liao *et al.*, 2008; Vilà *et al.*, 2011). These changes in ecosystem function have the potential to greatly alter ecosystem services (e.g., air and water purification) on which humans rely (Charles & Dukes, 2007; Eviner *et al.*, 2012).

Understanding how invasive plants influence soil nitrogen (N) availability is of particular importance. Nitrogen is a limiting nutrient for plant growth in most terrestrial ecosystems (Vitousek & Howarth, 1991; LeBauer & Treseder, 2008) and modifications to N availability may facilitate persistent invasive populations (Kulmatiski, 2006) and potentially inhibit successful re-vegetation of desired species (D'Antonio & Meyerson, 2002; Suding *et al.*, 2004b; Corbin & D'Antonio, 2012). Recent reviews by Ehrenfeld, (2003), Liao *et al.*, (2008), and Vilà *et al.*, (2011) suggest that invasive plants vary in their effects on N pools and processes, with some studies demonstrating increases and others showing decreases with invasion. For instance, Hawkes *et al.*, (2005) found that two exotic species, *Avena barbata* and *Bromus hordeaceous,* increased gross nitrification rates compared native species (*Nassella pulchra* and *Lupinus bicolor*), and Holly *et al.*, (2009) showed that litter of the exotic species, *Imperata cylindrical*, decomposed more rapidly than that of a native grass species (*Andropogon glomeratus)*. In contrast, Evans *et al.*, (2001) reported decreases in pools of inorganic soil N and potential rates of net N mineralization and denitrification associated with *Bromus tectorum* invasion*,* and Dassonville *et al.*, (2011) demonstrated reduced nitrification and denitrification potentials associated with the invasive species, *Fallopia japonica* and *Fallopia xbohemica*. Moreover, there are examples where the same invasive species (e.g., *Bromus tectorum*) has been shown to differentially affect soil N dynamics across studies (Bolton *et al.*, 1990; Evans *et al.*, 2001; Svejcar & Sheley, 2001; Sperry *et al.*, 2006; Schaeffer *et al.*, 2012). Variability among results, which may be due to differences in pre-invaded site dynamics (Suding *et al.*, 2004a; Kulmatiski *et al.*, 2006), exotic and native plant traits (Drenovsky *et al.*, 2012; Castro-Díez *et al.*, 2014), and time since establishment (Strayer *et al.*, 2006), highlight the complexity of ecosystem responses to invasion and demonstrate the need for continued research in this area.

Our study aimed to elucidate how two species invading California grasslands, *Aegilops triuncialis* (Barb goatgrass) and *Elymus caput-medusae* (Meduseahead), transform soil N cycling and availability. *A. triuncialis* is a winter annual native to Asia and Mediterranean Europe. It was introduced to the U.S. in the early 1900s (first sighting in 1914; Peters *et al.*, 1996), and continues to spread throughout California, Oregon, and Nevada (D'Antonio & Malmstrom, 2007). *E. caput-medusae* is also a winter annual native to Mediterranean Europe (Bossard *et al.*, 2000) that was introduced in the late 1800s to Oregon, and which quickly spread across the western U.S. (D'Antonio & Malmstrom, 2007). It continues to increase its range by 12% (in area) every year (Duncan *et al.*, 2005). Because *A. triuncialis* and *E. caput-medusae* are proving to be aggressive invaders (rated as 'high threats' by the California Invasive Plant Council), it is critical to understand the mechanisms by which they invade and the resulting effects on ecosystem structure and function.

Previous work has shown *A. triuncialis* and *E. caput-medusae* have traits that could affect soil N availability and rates of cycling compared to other species. For instance, reduced rates of litter decomposition have been observed with *A. triuncialis* (Eviner, 2004; Drenovsky & Batten, 2007) and *E. caput-medusae* (Bovey *et al.*, 1961) invasion, which may be due to high litter carbon to nitrogen (C:N; Eviner, 2004; Drenovsky & Batten, 2007) and lignin to N mass ratios (Drenovsky & Batten, 2007) in the case of *A. triuncialis*, and high silica content in the case of *E. caput-medusae* (Bovey *et al.*, 1961; Swenson *et al.*, 1964). Litter decomposition is an important mechanism by which available N (NH_4^+) is released into the soil (Chapman *et al.*, 2006; Parton *et al.*, 2007); consequently, species-specific alterations in litter quality and decomposition rates may translate into changes in N availability and rates of N cycling (Facelli & Pickett, 1991; Stump & Binkley, 1993).

In order to understand how *A. triuncialis* and *E. caput-medusae* affect soil N cycling, we examined N dynamics in three experimental grassland communities comprised of native perennials and annuals, naturalized exotic annuals, and *A. triuncialis* and *E. caput-medusae.* Naturalized exotics comprise a large portion of California grasslands, and native perennials are often the target community in restoration efforts; as such, these are two ecologically relevant plant communities by which to compare the effects of *A. triuncialis* and *E. caput-medusae* invasion. We hypothesized that *A. triuncialis* and *E. caput-medusae* would slow soil N cycling compared to native communities (H1) and naturalized communities (H2). To test this, we employed a combination of in-situ measurements and laboratory potential assays multiple times throughout the year. In-situ measurements (e.g., ion-exchange resin bags) provide insight into actual rates of N availability at a given time, while potential assays approximate the size of a specific microbial group (e.g., nitrifiers or denitrifiers). For nitrification (an obligate process), a potential assay approximates the likely rates experienced over longer periods of time (Hart *et al.*, 1994). We also measured the soil microclimate, total microbial biomass, and abundances of protozoa, in order to provide a mechanistic insight into the effects of *A. triuncialis* and *E. caput-medusae* on soil N cycling.

2.3 Methods

2.3.1 Site Description & Experimental Design

To test our hypotheses, we collected soil samples from an experimental site located in Davis, California (38°32’45.52”N, 121°47’05.37”W). The site elevation is 18.6 m and the climate is Mediterranean, with hot dry summers and cool wet winters. Mean annual temperature (MAT) is 15.7 °C. In 2011, summer (June – September) and winter (December – February) mean daily maximum air temperatures were 32.2 °C and 13.8 °C, respectively. In 2012, mean daily maximum air temperatures in summer were 33.3 °C and winter temperatures averaged 15.3 °C. Mean annual precipitation is 485 mm, with 75-100% of that occurring between November and April. In 2011 and 2012, annual precipitation was 173 mm and 216 mm, respectively. The soils at this site are primarily

(> 75% of the area) of the Reiff series (coarse-loamy, mixed, superactive, nonacid, thermic Mollic Xerofluvents); the other soil series present (< 25% of the area) is the Brentwood soil series (fine, smectitic, thermic Typic Haploxerepts) with a 0-2% slope (USDA Web Soil Survey, http://websoilsurvey.sc.egov.usda.gov).

In 2006, experimental grassland communities were established in an old agricultural field. Plots measuring 1.5 x 1.5 m each were arranged in a randomized complete block design and seeded as monocultures or mixtures of: (1) native species (*Bromus carinatus, Elymus glaucus, Elymus triticoides, Ascispon americanus, Lupinus bicolor, Stipa pulchra, Poa secunda*, and *Festuca microstachys*) (2) naturalized annual species (*Avena fatua, Bromus hordeaceus, Festuca multiflorum*, and *Trifolium subterraneum*) or (3) invasive winter annual species (*Aegilops triuncialis* and *Elymus caput-medusae*). Prior to seeding, the site was irrigated to stimulate germination of the existing seedbank within the soil; plants that germinated were sprayed with glyphosate. A total of 139 g of seed was added to each 1.5 x 1.5 m plot, with an equal proportion of each species in the mixture. Plant communities were allowed to diverge over time; however, non-planted species were removed periodically. For this study, we utilized three of the vegetation treatments: communities of native species, communities of naturalized species, and communities of invasive species. At the time of collection, N fixing species (*Acmispon americanus, Lupinus bicolor, Trifolium subterraneum*) were absent from all treatments and the native communities were dominated by perennial plants *Elymus glaucus* and *Stipa pulchra.*

2.3.2 Sample Collection

Mediterranean-type ecosystems experience strong seasonality in soil temperature, soil water content, and plant phenology, and thus the relative effects of plant community composition may vary considerably throughout the year. In order to account for this, we sampled in April 2011 and four times throughout 2011-12 during periods representative of the major seasons (October – warm and wet; January – cool and wet; April – warm and wet; July – hot and dry). Within each date, we sampled five soil cores (2.5 cm diameter x 15 cm deep) per plot and combined them into a single sample in order to account for within-plot spatial variability. For all soil analyses, there were 8 replicates per treatment (n = 8), except for April 2011 in which case there were 10 replicates (n = 10). Field moist soils were sieved (2 mm) and stored at 4 °C until analysis (the average time to analyze all variables was 1 week).

2.3.3 Soil Properties

Soil moisture was determined gravimetrically by drying the samples at 105°C until constant mass. For total carbon (C) and N analysis, we ground the soils to a fine powder and composited samples over the four sampling dates (October 2011 – July 2012) prior to analysis on an Elemental Combustion System (Costech Analytical Technologies Inc., Valencia, CA, USA). Samples were composited within each treatment because total C

and N were not expected to show strong seasonal variation (Binkley & Hart, 1989). Volumetric water content (VWC) was measured at each sampling date using a Time-Domain Reflectometer (TDR; MiniTrase, Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Fifteen-cm long TDR probes were inserted vertically into the soil in October 2011 and 30-cm long probes were installed adjacent to the 15-cm probes in January 2012 (n = 6/depth). This design allowed measurement of VWC within 0-15 cm and 15-30 cm soil layers. Soil pH was measured using a pH electrode (Orion DUAL STAR meter, Thermo Scientific, Waltham, MA, USA) after allowing 15 g fresh weight soil to equilibrate with 30 mL 0.01 $CaCl_2$ for 30 minutes (the soil slurry was mixed continuously during measurement). Soil temperature was measured at every sampling date using a traceable metal thermometer (Control Company, Friendswood, TX, USA) at 7.5 cm depth.

2.3.4 Nitrogen Availability

Soil available N was measured using two indices. First, static (instantaneous) inorganic (NH_4^+ and NO_3^-) pool sizes were measured four times (October, January, April, July) by extracting 15 g field-moist soil with 100 mL of 2 M KCl. Samples were shaken on a mechanical shaker for 1 hour, filtered using pre-leached Whatman No. 1 filter paper, and stored at -20 °C until analysis on a Lachat AE Flow Injection Autoanalyzer (Lachat Instruments, Inc., Loveland, CO, USA). In addition, ion-exchange (IER) resin bags were used to measure the accumulation of in-situ NH_4^+ and NO_3^- over time, expressed on a per g dry resin per day basis (Binkley & Matson, 1983; Binkley, 1984). Bags were prepared by measuring 15 mL (approximately 8 g wet weight and 4 g oven-dry equivalent) of cation and anion resin beads (J.T. Baker Mixed Bed Exchange Resin, IONAC NM-60 H^+/OH^- Form, Type 1, 16-50 Mesh) into undyed nylon stockings that were subsequently tied shut. One bag per plot was buried at a depth of 7.5 cm and incubated between 5 and 7 weeks. After the end of the allotted incubation time, each resin bag was removed and exchanged with a new one (using the same hole at 7.5 cm depth). The first bags were buried on October 18, 2011 and the last bags were removed on August 31, 2012, totaling eight incubations. Some of the removal and insertion dates corresponded with soil sampling dates. Once removed from the field, resin bags were immediately air-dried, weighed, and placed into 125 mL flasks. To each flask, 50 mL of 2M KCl was added. Samples were then shaken uncovered for 1 hour on an orbital shaker, filtered, and stored at -20°C until analysis of filtrate on a Lachat AE Flow Injection Autoanalyzer.

2.3.5 Potential Assays and Microbial Biomass

Potential rates of nitrification were measured using a shaken soil-slurry method as described by (Hart *et al.*, 1994). By creating optimal conditions for nitrification, this 24-h incubation provides an approximation of the population size of nitrifying microorganisms in the soil. Briefly, 100 mL of a solution containing 1.5 mM of NH_4^+ and 1 mM of PO_4^{3-} (pH = 7.2) was added to 15 g field-moist soil in a 250 mL flask. Flasks were capped with a rubber stopper containing a hole and placed on an orbital shaker at 180 rpm for 24

hours. Sampling occurred at 2, 4, 22, and 24 hours by removing a 10 mL aliquot of the suspension from each flask and centrifuging at 8,000 x g for 8 minutes. Five mL of supernatant was then placed into a disposable polypropylene tube, capped, and stored at -20°C until analysis for NO_3^- on a Lachat AE Flow Injection Autoanalyzer. Concentrations of NH_4^+ were also analyzed from these samples in order to check that nitrification never became limited by NH_4^+.

Similar to nitrification potential, denitrification potential is an index of the size of the denitrifying microbial community and the pool of denitrifying enzymes. We measured denitrification potential by modifying a protocol developed by (Smith & Tiedje, 1979). Briefly, non-limiting conditions were created by amending 50 g field-moist soil with NO_3^- and labile C (0.1 mg N-NO_3^- g^{-1} soil, 1 mg C-glucose g^{-1} soil, and 1 mg C-glutamic acid g $soil^{-1}$) in a 250 mL flask. The flasks were sealed tight with a rubber stopper and septum and 20 mL of acetylene was added in order to inhibit the reduction of N_2O to N_2. The soils were incubated anaerobically on an orbital shaker (180 rpm) for 90 minutes; 15 mL of the headspace was sampled at 30 and 90 minutes. Gas samples were stored in evacuated Exetainer® until analysis for N_2O production on a Shimadzu GC-2014 electron capture detector (Shimadzu Corporation, Columbia, MD, USA). Values of N_2O produced were used to estimate the overall size of the denitrifying microbial community.

Microbial biomass was estimated using three methods that provide complementary information: substrate induced respiration (SIR; measured at all 4 time points from October 2011 – July 2012), chloroform fumigation-extraction (CFE; measured in January 2012), and microscopic enumeration (measured in April 2011; n = 10). By measuring CO_2 production, substrate induced respiration estimates those microorganisms that are active and glucose-responsive (Wardle & Ghani, 1995). The chloroform fumigation extraction method more closely captures total microbial biomass and provides information on biomass N (Martikainen & Palojärvi, 1990; Bailey *et al.*, 2002), while direct enumeration allows for the separate quantification of bacteria and fungi (Paul *et al.*, 1999).

Substrate induced respiration was modified from (West & Sparling, 1986). Fifteen g of field-moist soil was weighed out into 250 mL flasks and 30 mL of a glucose solution (30 mg glucose ml^{-1} H_2O) was added to each. The flasks were sealed tight with a rubber stopper and septum and placed on a shaker (180 rpm) for 2.5 hours. At 0.5, 1.5, and 2.5 hours, 15 mL of the headspace was sampled for CO_2 and stored in evacuated Exetainer® until analysis on a on a Shimadzu GC-2014 thermal conductivity detector. Changes in [CO_2] over time were used to calculate microbial biomass (West & Sparling, 1986). The chloroform fumigation-extraction method was used to estimate microbial biomass carbon (C) and N (Haubensak *et al.*, 2002). Microbial C and N were calculated by dividing the fumigation C-flush and N-flush by a k_{EC} factor of 0.49 (Joergensen, 1996) and 0.68 (Brookes *et al.*, 1985), respectively. Direct enumeration of total bacteria and fungi was conducted using microscopy. Samples (10 g) were shipped overnight on dry ice to Soil Food Web, Inc. (Corvallis OR, USA) where total bacteria was estimated by direct

counting using a fluorescein isothiocyanate (FITC) method (Babiuk & Paul, 1970) and fungal biomass was calculated by measuring the diameter and length of hyphae (Lodge & Ingham, 1991). The Soil Food Web Inc. also estimated abundance of protozoa in each sample. Protozoa can be categorized into three subgroups depending on size: flagellates, and amoeba. Subgroups were differentiated and enumerated by direct counting of serial dilutions using microscopy. The direct counts were then used to estimate total protozoa population sizes using the most probable number approach (Darbyshire *et al.*, 1974).

2.3.6 Statistical Analysis

Repeated measures analysis of variance (RMANOVA) was used to assess significant effects of treatment ("plant community type") and date on instantaneous and accumulated (IER bag) inorganic N pools, nitrification and denitrification potential, microbial biomass as measured by SIR, soil water content, and temperature. A one-way ANOVA was used to assess significant differences in protozoan biomass and microbial biomass as determined by CFE and direct enumeration because they were sampled only once. Soil NO_3^- and NH_4^+pools, IER- NO_3^- and NH_4^+, and volumetric water content (0-15 cm) were log transformed in order to fulfill the assumptions of an ANOVA. In addition, two IER-NO_3^- outliers (values > 3 SD of mean) were removed. A post-hoc Tukey's test ($\alpha = 0.05$) was used to make comparisons between treatment means when the ANOVA model was significant. Potential nitrification and denitrification were correlated using Pearson's product moment correlation coefficient, as were volumetric and gravimetric water content.

We used bidirectional stepwise multiple regression in order to explore potential relationships among potential rates and biotic (e.g., microbial biomass) and abiotic (e.g., inorganic N, soil moisture) variables (Table 3). When necessary, data was transformed to meet the assumption of normality. All statistical analyses were performed in 'R' statistical software (www.r-project.org).

2.4 Results

2.4.1 Nitrogen Availability

Pools of soil NH_4^+ varied by date but not plant community type (RM ANOVA; plant community type, $P = 0.22$; date, $P < 0.001$; community x date, $P = 0.54$). Soil NO_3^- pools were also affected by date and were smaller in naturalized treatments compared to native treatments, although this effect was only marginally significant (RM ANOVA; plant community type, $P = 0.06$; date, $P < 0.001$; community x date, $P = 0.5$). Total inorganic N (TIN; NH_4^+ and NO_3^-) was affected by plant community type and date (RM ANOVA; plant community type, $P = 0.04$; date, $P < 0.001$; community x date, $P = 0.82$); however, when analyzed using a post hoc analysis, naturalized ($P = 0.08$) and invaded (0.06) treatments were only marginally reduced compared to native treatments.

Ion-exchange resin bags are likely a more sensitive measure of N availability than pools of soil N, given that the buried bags capture N accumulated in soil over time and pools of N are instantaneous measurements. Indeed, soil IER-NH_4^+ was significantly affected by plant community type and date (Figure 2-2, RM ANOVA; plant community type, $P = 0.03$; date, $P < 0.001$; community x date, $P = 0.61$). Specifically, invaded treatments had lower IER-NH_4^+ values than native treatments ($P = 0.02$). Plant community type and date had significant interactive effects on IER-NO_3^- (Figure 2-2, RM ANOVA; plant community type, $P < 0.001$; date, $P < 0.001$; community x date, $P = 0.02$), with invaded treatments exhibiting lower IER-NO_3^- than native treatments in April ($P = 0.04$). Total available IER-N (NH_4^+ and NO_3^-) was affected by plant community type, independent of date (RM ANOVA; plant community type, $P < 0.001$; date, $P < 0.001$; community x date, $P = 0.11$). Here, native treatments had higher values of IER-N than invaded treatments ($P < 0.001$) and naturalized communities had intermediate values (Naturalized – Invasive, $P = 0.04$; Naturalized – Native, $P = 0.04$).

2.4.2 Potential Rates of Nitrification, Denitrification, and Microbial Biomass

Plant community type and date had significant independent effects on potential nitrification rates (Figure 2-3, RM ANOVA; plant community type, $P < 0.001$; date, $P < 0.001$; community x date, $P = 0.31$). Potential nitrification was significantly lower in naturalized ($P < 0.001$) and invaded ($P < 0.001$) treatments than native treatments. Community effects on potential nitrification were correlated with soil moisture and microbial biomass for all dates except April 2011 and 2012 (Table 2-4), in which case potential nitrification was not explained by any of the variables included in the model (Table 2-3).

Potential denitrification was affected independently by plant community type and date (Figure 2-3, RM ANOVA; plant community type, $P < 0.001$; date, $P < 0.001$; community x date, $P = 0.41$), following a similar pattern to potential nitrification over time (lowest values in October and April, highest values in January and July; $R = 0.68$, $P = 0.03$). Rates of potential denitrification were significantly reduced in invaded ($P < 0.001$) and naturalized plots ($P = 0.004$) compared to native plots. Changes in potential denitrification were closely associated with microbial biomass during the growing season (October – April) and were correlated with both microbial biomass and soil moisture during the summer (Table 2-4).

Microbial biomass as measured by SIR was significantly affected by plant community composition (Figure 2-3, RM ANOVA; plant community type, $P < 0.001$; date, $P < 0.001$; community x date, $P = 0.46$). Values were significantly reduced in invaded plots compared to native plots and naturalized communities had intermediate values. SIR varied significantly over time, with the highest values occurring during July.

Total fungi and bacteria, measured using direct enumeration in April 2011, did not show significant differences among plant community type (Table 2-2, ANOVA; total bacteria, P = 0.84; total fungi, P = 0.50). However, microbial C, which was measured by CFE, was significantly reduced in naturalized (Table 2-2, ANOVA; P = 0.002) and invaded communities (P = 0.04) compared to native. Microbial N was also affected by plant community type (ANOVA; P = 0.05). Naturalized communities had significantly lower microbial N than native communities (P = 0.04); invaded plots did not differ from either native (P = 0.22) or naturalized (P = 0.60) plots.

Soils from April 2011 were analyzed for biomass within each subgroup of protozoa (Figure 2-4). Across all treatments, amoeba dominated the soils (mean = 17.5 organisms kg^{-1} soil), followed by flagellates (mean = 7.8 organisms kg^{-1} soil), and finally ciliates (mean = 0.4 organisms kg^{-1} soil). Plant community type had no significant effect on the abundance of flagellates (ANOVA; P = 0.25) and ciliates (P = 0.61); naturalized plots had higher amounts of amoeba per kg of soil than invaded treatments, although this difference was only marginally significant (P = 0.06)

2.4.3 Soil Properties

Plant community type and date had significant, independent effects on volumetric soil water content at 0-15 cm depth (Figure 2-1, RM ANOVA model; plant community type, P < 0.001; date, P < 0.001; community x date, P = 0.16) and 15-30 cm depth (RM ANOVA model; plant community type, P <0.001; date, P< 0.001; community x date = 0.81). Volumetric water content was also affected by soil depth (P < 0.001); on average, shallower soils (0-15 cm) had less soil moisture than deeper soils (15-30 cm), although differences by depth were dependent on time (P = 0.006). Gravimetric and volumetric water content within the top 15 cm of the soil profile were positively correlated (r = 0.76, P < 0.001). Despite this, gravimetric soil water content (0-15 cm depth) was statistically similar among plant community type, indicating that it was a less sensitive measure of community effects. Gravimetric water content did, however, differ by date (RM ANOVA model; plant community type, P = 0.14; date, P < 0.001; community x date, P = 0.40). Soil pH and temperature were significantly affected by date but not by treatment (Table 2-1, RM ANOVA; pH: plant community type, P = 0.95; date, P < 0.001; community x date, P = 0.80; temperature: plant community type, P = 0.91; date, P < 0.001, community x date, P = 0.32). There were no significant effects of plant community type on soil total C, total N, or C/N ratio (Table 2-1, ANOVA; P = 0.251, P = 0.184, P = 0.843, respectively).

2.5 Discussion

Our study demonstrated that *A. triuncialis* and *E. caput-medusae* communities had significantly lower rates of N cycling than native communities (H1). These findings are similar to some studies comparing native perennials and exotic annuals (Evans *et al.*, 2001; Corbin & D'Antonio, 2011), but contrast with others that demonstrated an increase

in N cycling associated with exotic annual invasion (Hawkes *et al.*, 2005; Parker & Schimel, 2010) or no differences between the two community types (Bolton *et al.*, 1990; Svejcar & Sheley, 2001). In our study, reduced inorganic N availability and potential rates of nitrification and denitrification associated with invasion may be due, in part, to lower decomposition rates. Prior work at our site showed that native plants had significantly higher litter N concentrations than *A. triuncialis* and *E. caput-medusae* (Baty, 2012), which could result in higher rates of decomposition and N mineralization in native communities. Lower litter quality and reduced decomposition rates of *A. triuncialis* and *E. caput-medusae* have been demonstrated elsewhere (Bovey *et al.*, 1961; Swenson *et al.*, 1964; Eviner, 2004; Drenovsky & Batten, 2007) supporting this as a possible mechanism by which N cycling is altered in this system.

While lower N availability was observed in invaded compared to native communities (as measured by IER bags), neither changes in NH_4^+ nor NO_3^- availability could account for reductions in nitrification and denitrification potentials (based on a multiple stepwise regression; Table 2-4). This finding is similar to Hawkes *et al.*, (2005) where NH_4^+ concentrations failed to predict increases in gross nitrification with invasion. The lack of relationship between substrate availability and potential rates is surprising, given that actual rates of denitrification and nitrification should be strongly regulated by NO_3^- and NH_4^+ supply (Booth *et al.*, 2005). However, Wallenstein *et al.*, (2006) argue that NO_3^- availability has little effect on denitrifying microbial community structure and abundance, and thus potential rates, even though availability can affect actual in-situ rates of denitrification. Moreover, Horz *et al.*, (2004) note that significant changes in NH_4^+ may be required to stimulate growth of ammonia oxidizing microorganisms; thus, it is possible that nitrifying microbial abundance and potential rates are insensitive to changes in NH_4^+ produced by alterations in plant community composition, and are instead regulated by other factors such as soil moisture availability.

Indeed, changes in potential nitrification and denitrification with invasion were best explained by reductions in soil moisture and total microbial biomass (SIR) at 0-15 cm depth (based on a multiple stepwise regression; Table 2-4). Specifically, nitrification potential was explained by soil moisture and total microbial biomass on all dates except April, in which case soil moisture and microbial biomass ceased to explain any variation. In addition, changes in denitrification potential with exotic plant invasion were influenced by shifts in total microbial biomass. Soil moisture was also a significant explanatory variable in July, with potential denitrification showing a positive response to increases in soil moisture. These results demonstrate that *A. triuncialis* and *E. caput-medusae* altered soil moisture availability and total microbial biomass compared to native communities, likely because annuals have shallower roots than perennials (Holmes & Rice, 1996). As a result, invaded communities had lower nitrification and denitrification potentials than native communities.

The disparity between N cycling in native and invaded soils may have implications for restoration. Native perennials are often used to re-vegetate sites after the removal of

invasive species (Stromberg *et al.*, 2007); if legacies of altered N cycling persist after the removal of *A. triuncialis* or *E. caput-medusae*, as has been shown with other invasive plants (Corbin & D'Antonio, 2004; 2012), the competitive edge may be shifted away from natives and successful reestablishment may be hindered (Gorman & Suding, 2010). While *A. triuncialis* can reduce the success of native species by altering the soil microbial community (Batten *et al.*, 2008), additional studies are needed in order to determine whether the same is true for alterations in soil N cycling by both *A. triuncialis* and *E. caput-medusae.*

In contrast to our second hypothesis, N cycling was not lower in invaded relative to naturalized plant communities (H2). This may be because the effect of an invasive plant is likely linked to how functionally distinct it is from the resident species (Corbin & D'Antonio, 2011). Thus, differences in important morphological or physiological characteristics between these two groups might not be large enough to induce changes in N cycling. For example, *A. triuncialis* and *E. caput-medusae* have similar litter C:N ratios as *Bromus hordeaceus* (Eviner, 2004) which dominated naturalized plots at the time of sampling. Additionally, invasive and naturalized exotics utilized in this study are all annuals and may consequently share similar rooting depths (as suggested by similarities in volumetric water content at 0-15 and 15-30 cm depths; Figure 2-1), and exploit N in similar ways. Additional studies explicitly looking at rooting depths and N use between these two communities are needed to confirm this hypothesis.

The similarity in N cycling between invaded and naturalized soils has at least two implications. First, alterations to N availability may not be a mechanism by which *A. triuncialis* and *E. caput-medusae* establish persistent populations in naturalized grasslands of California. Instead, changes to N cycling may be a "byproduct" of other mechanisms responsible for the success of these species. For instance, dense layers of *E. caput-medusae* litter have been shown to favor the emergence of *E. caput-medusae* seedlings while inhibiting the germination of other species, such as *Convolvulus arvensis* (Evans & Young, 1970). Second, the invasion of naturalized grasslands by *A. triuncialis* and *E. caput-medusae* may not result in altered soil N dynamics.

2.6 References

Babiuk LA, Paul EA (1970) The use of fluorescein isothiocyanate in the determination of the bacterial biomass of grassland soil. *Canadian Journal of Microbiology*, **17**, 57–62.

Bailey VL, Peacock AD, Smith JL, Bolton H (2002) Relationships between soil microbial biomass determined by chloroform fumigation–extraction, substrate-induced respiration, and phospholipid fatty acid analysis. *Soil Biology and Biochemistry*, **34**, 1385–1389.

Batten KM, Scow KM, Espeland EK (2008) Soil microbial community associated with an invasive grass differentially impacts native plant performance. *Microbial Ecology*.

Baty JH (2012) Changes to the Seasonality of Plant-Soil Systems by Three Phenologically Distinct Groups of California Grassland Plants. *University of California Davis*.

Binkley D (1984) Ion Exchange Resin Bags: Factors Affecting Estimates of Nitrogen Availability1. *Soil Science Society of America Journal*, **48**, 1181–1184.

Binkley D, Hart SC (1989) The components of nitrogen availability assessments in forest soils. In: *Advances in Soil Science* pp57–112. New York, Springer-Verlag.

Binkley D, Matson P (1983) Ion exchange resin bag method for assessing forest soil nitrogen availability. *Soil Science Society of America Journal,* **47**, 1050–1052.

Bolton H, Wildung RE, Smith JL (1990) Nitrogen mineralization potentials of shrub-steppe soils with different disturbance histories. *Soil Science Society of America Journal*, **54**, 887–891.

Booth MS, Stark JM, rastetter E (2005) Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecological Monographs*, **75**, 139–157.

Bossard CC, Randall JM, Hoshovsky MC (Eds.) (2000) *Invasive plants of California's wildlands*. Berkeley, University of California Press.

Bovey RW, Le Tourneau D, Erickson LC (1961) The chemical composition of medusahead and downy brome. *Weeds*, **9**, 307–311.

Bradley BA, Blumenthal DM, Early R, et al. (2012) Global change, global trade, and the next wave of plant invasions. *Frontiers in Ecology and the Environment*, **10**, 20–28.

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry*, **17**, 837–842.

Castro-Díez P, Godoy O, Alonso A, Gallardo A, Saldaña A (2014) What explains variation in the impacts of exotic plant invasions on the nitrogen cycle? A meta-analysis. *Ecology Letters*, **17**, 1–12.

Chapman SK, Langley JA, Hart SC, Koch GW (2006) Plants actively control nitrogen cycling: uncorking the microbial bottleneck. *New Phytologist*, **169**, 27–34.

Charles H, Dukes JS (2007) Impacts of invasive species on ecosystem services. In: *Biological invasions* pp217–237. Springer.

Corbin JD, D'Antonio CM (2004) Effects of exotic species on soil nitrogen cycling: Implications for restoration. *Weed Technology*, **18**, 1464–1467.

Corbin JD, D'Antonio CM (2011) Abundance and productivity mediate invader effects on nitrogen dynamics in a California grassland. *Ecosphere*, **2**, art32.

Corbin JD, D'Antonio CM (2012) Gone but Not Forgotten? Invasive Plants' Legacies on Community and Ecosystem Properties. *Invasive Plant Science and Management*, **5**, 117–124.

D'Antonio CM, Malmstrom C, Reynolds SA, Gerlach J (2007) Ecology of invasive non-native species in California grassland. In: *California Grasslands: Ecology and Management* Stromberg MR, Corbin JD, D'Antonio C pp67–87. Berkeley, University of California Press.

Darbyshire JF, Wheatley RE, Greaves MP, Inkson RHE (1974) A rapid micromethod for estimating bacterial and protozoan populations in soil. *Ecology*, **61**, 764–771.

Dassonville N, Guillaumaud N, Piola F, Meerts P, Poly F (2011) Niche construction by the invasive Asian knotweeds (species complex Fallopia): impact on activity, abundance and community structure of denitrifiers and nitrifiers. *Biological Invasions*, **13**, 1115–1133.

Davidson AM, Jennions M, Nicotra AB (2011) Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters*, **14**, 419–431.

Drenovsky RE, Batten KM (2007) Invasion by Aegilops triuncialis (Barb Goatgrass) Slows Carbon and Nutrient Cycling in a Serpentine Grassland. *Biological Invasions*, **9**, 107–116.

Drenovsky RE, Grewell BJ, D'Antonio CM, et al. (2012) A functional trait perspective on plant invasion. *Annals of Botany*, **110**, 141–153.

Duncan CA, Clark JK, Duncan CA, Clark JK (2005) *Invasive plants of range and wildlands and their environmental, economic and societal impacts.* Weed Science Society of America.

D'Antonio C, Meyerson LA (2002) Exotic Plant Species as Problems and Solutions in Ecological Restoration: A Synthesis. *Restoration Ecology*, **10**, 703–713.

Ehrenfeld JG (2003) Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems*, **6**, 503–523.

Evans RA, Young JA (1970) Plant litter and establishment of alien annual weed species in rangeland communities. *Weed Science*, **18**, 697–703.

Evans RD, Rimer R, Sperry L, Belnap J (2001) Exotic plant invasion alters nitrogen dynamics in an arid grassland. *Ecological Applications*, **11**, 1301–1310.

Eviner VT (2004) Plant traits that influence ecosystem processes vary independently among species. *Ecology*, **85**, 2215–2229.

Eviner VT, Garbach K, Baty JH, Hoskinson SA (2012) Measuring the Effects of Invasive Plants on Ecosystem Services: Challenges and Prospects. *Invasive Plant Science and Management*, **5**, 125–136.

Facelli JM, Pickett ST (1991) Plant litter: its dynamics and effects on plant community structure. *The Botanical Review*, **57**, 1–32.

Grman E, Suding KN (2010) Within-Year Soil Legacies Contribute to Strong Priority Effects of Exotics on Native California Grassland Communities. *Restoration Ecology*, **18**, 664–670.

Hart SC, Stark JM, Davidson EA, Firestone MK (1994) Nitrogen mineralization, immobilization, and nitrification. *Methods of Soil Analysis: Part 2—Microbiological and Biochemical Properties*, 985–1018.

Haubensak KA, Hart SC, Stark JM (2002) Influences of chloroform exposure time and soil water content on C and N release in forest soils. *Soil Biology and Biochemistry,* **34**, 1549–1562.

Hawkes CV, Wren IF, Herman DJ, Firestone MK (2005) Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecology Letters*, **8**, 976–985.

Holly DC, Ervin GN, Jackson CR, Diehl SV, Kirker GT (2009) Effect of an invasive grass on ambient rates of decomposition and microbial community structure: a search for causality. *Biological Invasions*, **11**, 1855–1868.

Holmes TH, Rice KJ (1996) Patterns of growth and soil-water utilization in some exotic annuals and native perennial bunchgrasses of California. *Annals of Botany*, **78**, 233–243.

Horz H-P, Barbrook A, Field CB, Bohannan BJM (2004) Ammonia-oxidizing bacteria respond to multifactorial global change. *Proceedings of the National Academy of Sciences*, **101**, 15136–15141.

Joergensen RG (1996) The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k EC value. *Soil Biology and Biochemistry*, **28**, 25–31.

Kulmatiski A (2006) Exotic plants establish persistent communities. *Plant Ecology*, **187**, 261–275.

Kulmatiski A, Beard KH, Stark JM (2006) Soil history as a primary control on plant invasion in abandoned agricultural fields. *Journal of Applied Ecology*, **43**, 868–876.

LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, **89**, 371–379.

Liao C, Peng R, Luo Y, et al. (2008) Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. *New Phytologist*, **177**, 706–714.

Lodge DJ, Ingham ER (1991) A comparison of agar film techniques for estimating fungal biovolumes in litter and soil. *Agriculture, Ecosystems & Environment*, **34**, 131–144.

Mack RN, Simberloff D, Mark Lonsdale W, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**, 689–710.

Martikainen PJ, Palojärvi A (1990) Evaluation of the fumigation-extraction method for the determination of microbial C and N in a range of forest soils. *Soil Biology and Biochemistry*, **22**, 797–802.

Parker SS, Schimel JP (2010) Invasive Grasses Increase Nitrogen Availability in California Grassland Soils. *Invasive Plant Science and Management*, **3**, 40–47.

Parton W, Silver WL, Burke IC, et al. (2007) Global-Scale Similarities in Nitrogen Release Patterns During Long-Term Decomposition. *Science*, **315**, 361–364.

Paul EA, Harris D, Klug MJ (1999) The determination of microbial biomass. In: *Standard Soil Methods for Long-Term Ecological Research* Robertson GP, Coleman DC, Bledsoe CS pp291–317. New York, Oxford University Press.

Peters A, Johnson DE, George MR (1996) Barb goatgrass: a threat to California rangelands. *Rangelands*, **18**, 8–10.

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, **52**, 273–288.

Sala OE, Chapin FS, Armesto JJ, et al. (2000) Global biodiversity scenarios for the year 2100. *Science*, **287**, 1770–1774.

Schaeffer SM, Ziegler SE, Belnap J, Evans RD (2012) Effects of Bromus tectorum invasion on microbial carbon and nitrogen cycling in two adjacent undisturbed arid grassland communities. *Biogeochemistry*, **111**, 427–441.

Smith MS, Tiedje JM (1979) Phases of denitrification following oxygen depletion in soil. *Soil Biology and Biochemistry*, **11**, 261–267.

Sperry LJ, Belnap J, Evans RD (2006) Bromus tectorum invasion alters nitrogen dynamics in an undisturbed arid grassland ecosystem. *Ecology*, **87**, 603–615.

Strayer DL, Eviner VT, Jeschke JM, Pace ML (2006) Understanding the long-term effects of species invasions. *Trends in Ecology & Evolution*, **21**, 645–651.

Stromberg MR, D'Antonio CM, Young TP, Wirka J, Kephart PR (2007) California grassland restoration. In: *California grasslands: Ecology and management*Stromberg MR, Corbin JD, D'Antonio CM pp254–280. Berkeley, University of California Press.

Stump LM, Binkley D (1993) Relationships between litter quality and nitrogen availability in Rocky Mountain forests. *Canadian Journal of Forest Research*, **23**, 492–502.

Suding K, LeJeune K, Seastedt T (2004a) Competitive impacts and responses of an invasive weed: dependencies on nitrogen and phosphorus availability. *Oecologia*, **141**.

Suding KN, Gross KL, Houseman GR (2004b) Alternative states and positive feedbacks in restoration ecology. *Trends in Ecology & Evolution*, **19**, 46–53.

Svejcar T, Sheley R (2001) Nitrogen dynamics in perennial- and annual-dominated arid rangeland. *Journal of arid environments*, **47**, 33–46.

Swenson CF, Le Tourneau D, Erickson LC (1964) Silica in medusahead. *Weeds*, **12**, 16–18.

Tylianakis JM, Didham RK, Bascompte J, Wardle DA (2008) Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, **11**, 1351–1363.

Vilà M, Espinar JL, Hejda M, et al. (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters*, **14**, 702–708.

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry*, **13**, 87–115.

Vitousek PM, D'Antonio CM, Loope LL, Rejmanek M, Westbrooks R (1997) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, **21**, 1–16.

Vitousek PM, Walker LR, Whiteaker LD, Mueller-Dombois D, Matson PA (1987) Biological Invasion by Myrica faya Alters Ecosystem Development in Hawaii. *Science*, **238**, 802–804.

Wallenstein MD, Myrold DD, Firestone M, Voytek M (2006) Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. *Ecological Applications*, **16**, 2143–2152.

Wardle DA, Ghani A (1995) Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? *Soil Biology and Biochemistry*, **27**, 821–828.

West AW, Sparling GP (1986) Modifications to the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents. *Journal of Microbiological Methods*, **5**, 177–189.

Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E (1998) Quantifying threats to imperiled species in the United States. *BioScience*, **48**, 607–615.

2.7 Tables

Table 2-1 Soil characteristics from 0-15 cm depth associated with each plant community type

Treatment	GWC (%)^	pH[#]	Temperature*	Total C (g kg^{-1} soil)	Total N (g kg^{-1} soil)	C:N[ϕ]
Native	14.3 (1.1)	6.6 (0.03)	18.5 (1.3)	12.9 (0.1)	1.34 (0.01)	9.63 (0.02)
Naturalized	13.9 (1.1)	6.6 (0.02)	18.4 (1.3)	12.3 (0.1)	1.28 (0.01)	9.59 (0.03)
Invaded	14.4 (1.0)	6.6 (0.02)	18.1 (1.3)	12.1 (0.1)	1.27 (0.01	9.55 (0.05)

^Gravimetric water content, [#]pH measured in 0.01 M CaCl2 solution, *Temperature measured at 7.5 cm depth, [ϕ]Carbon to nitrogen mass ratio. Note: Values are means ± SE averaged over all four seasons (October 2011 – July 2012; n = 8). The absence of letters signifies that no significant differences occurred among treatments ($P < 0.05$).

Table 2-2 Comparison of direct count measurements and chloroform fumigation extraction as affected by plant community type

	Direct Counts: April 2011			**CFE: January 2012**	
	Total Fungi	Total Bacteria	Microbial Biomass	Microbial Biomass	
Treatment		mg C kg^{-1} soil		mg C kg^{-1} soil	mg N kg^{-1} soil
Native	181.85 (10.76)	344.40 (41.45)	526.25 (43.37)	277.32 (10.22)[a]	37.67 (3.03)[a]
Naturalized	163.25 (14.24)	350.35 (69.28)	513.60 (77.66)	219.66 (9.18)[b]	29.96 (1.76)[b]
Invaded	187.30 (20.15)	378.60 (34.52)	569.90 (43.26)	240.09 (12.83)[b]	32.33 (2.19)[a]

Note: Values are means ± SE (n = 8). Different letters denote significant differences among plant community type within a given microbial characteristic (determined by a Tukey post-hoc test [$P < 0.05$]). If letters are absent, no significant differences were present.

Table 2-3 Soil and microbial characteristics used in stepwise regression to account for plant community effects on nitrification and denitrification potentials

Dependent variable	Independent variable
April 2011	
Nitrification potential	Soil moisture
	Fungi:bacteria ratio
	Flagellates
	Amoeba
	Ciliates
	C:N ratio
October 2011 - July 2012	
Nitrification potential	Soil temperature
	Soil moisture
	Soil pH
	Microbial biomass
	NH_4^+ pool size
	IER - NH_4^+
Denitrification potential	Soil temperature
	Soil moisture
	Soil pH
	Microbial biomass
	NO_3^- pool size
	IER - NO_3^-

Note: IER-NH_4^+/NO_3^- are changes in NH_4^+ and NO_3^- over time as captured by ion-exchange resin bags (buried at 7.5 cm depth).

Table 2-4 Stepwise multiple regression relating potential nitrification and denitrification to selected soil and microbial characteristics

Parameter	Regression coefficient	P
October 2011		
Nitrification potential: R^2 for full model = 0.33, P value = 0.02		
Soil moisture	0.35	0.08
Microbial biomass	0.01	0.02
Denitrification potential: R^2 for full model = 0.37, P value = $1.6x10^{-3}$		
Microbial biomass	0.13	$1.6x10^{-3}$
January 2012		
Nitrification potential: R^2 for full model = 0.60, P value = $7.08x10^{-5}$		
Soil moisture	-0.06	$7.7x10^{-3}$
Microbial biomass	0.01	$1.1x10^{-4}$
Denitrification potential: R^2 for full model = 0.27, P value = 0.01		
Microbial biomass	0.01	0.01
April 2012		
Denitrification potential: R^2 for full model = 0.27, P value = $9.4x10^{-3}$		
Microbial biomass	0.10	$9.4x10^{-3}$
July 2012		
Nitrification potential: R^2 for full model = 0.47, P value = $1.3x10^{-3}$		
Soil moisture	0.4	0.15
Microbial biomass	0.03	$5.9x10^{-4}$
Denitrification potential: R^2 for full model = 0.31, P value = 0.02		
Microbial biomass	0.12	0.02
Soil moisture	3.6	0.08

Note: Results are presented only for overall models found to be significant ($P < 0.05$). Nitrification for October was power transformed. Potential denitrification and nitrification were log transformed for January.

2.8 Figures

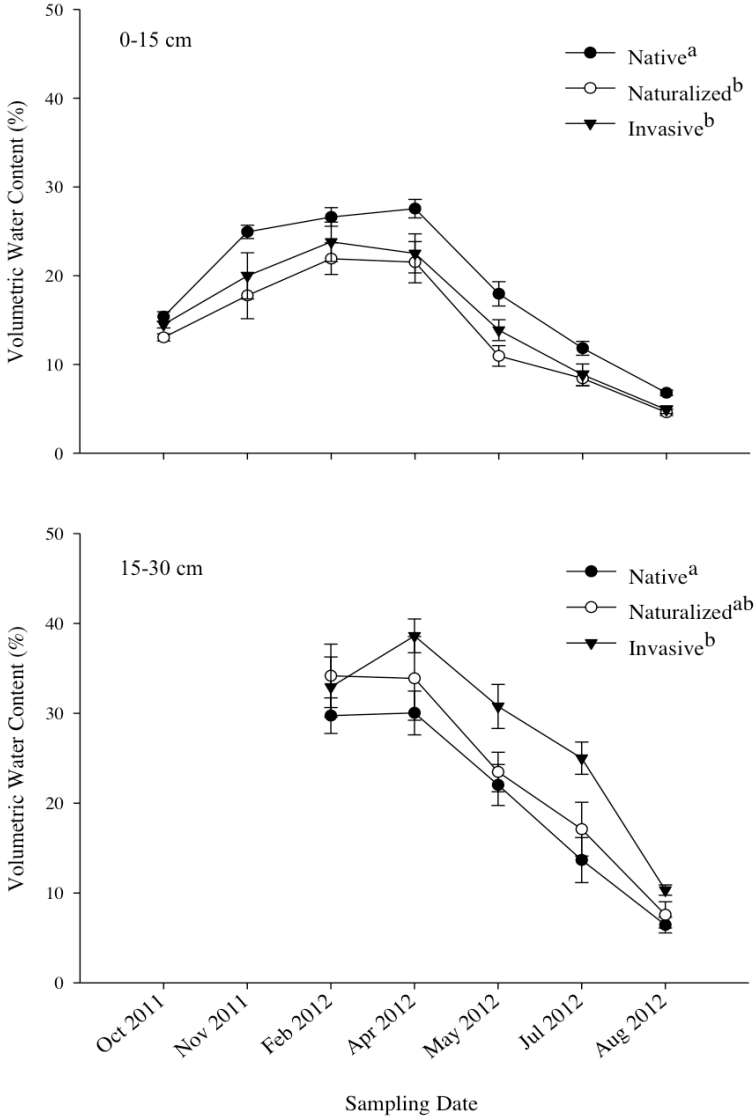

Figure 2-1 Changes in mean soil volumetric water content over time as influenced by plant community type (native, naturalized, and invasive) and soil depth (upper panel 0-15 cm, lower panel 15-30 cm). Values are means ± 1 SE (untransformed; n = 6). Different letters denote significant differences by plant community type at each depth (determined by a Tukey post-hoc test [$p < 0.05$]). Interactions between plant community type and date were nonsignificant. VWC 15-30 cm values are not presented for October and November 2011 because 0-30 cm TDR probes were not installed at that time.

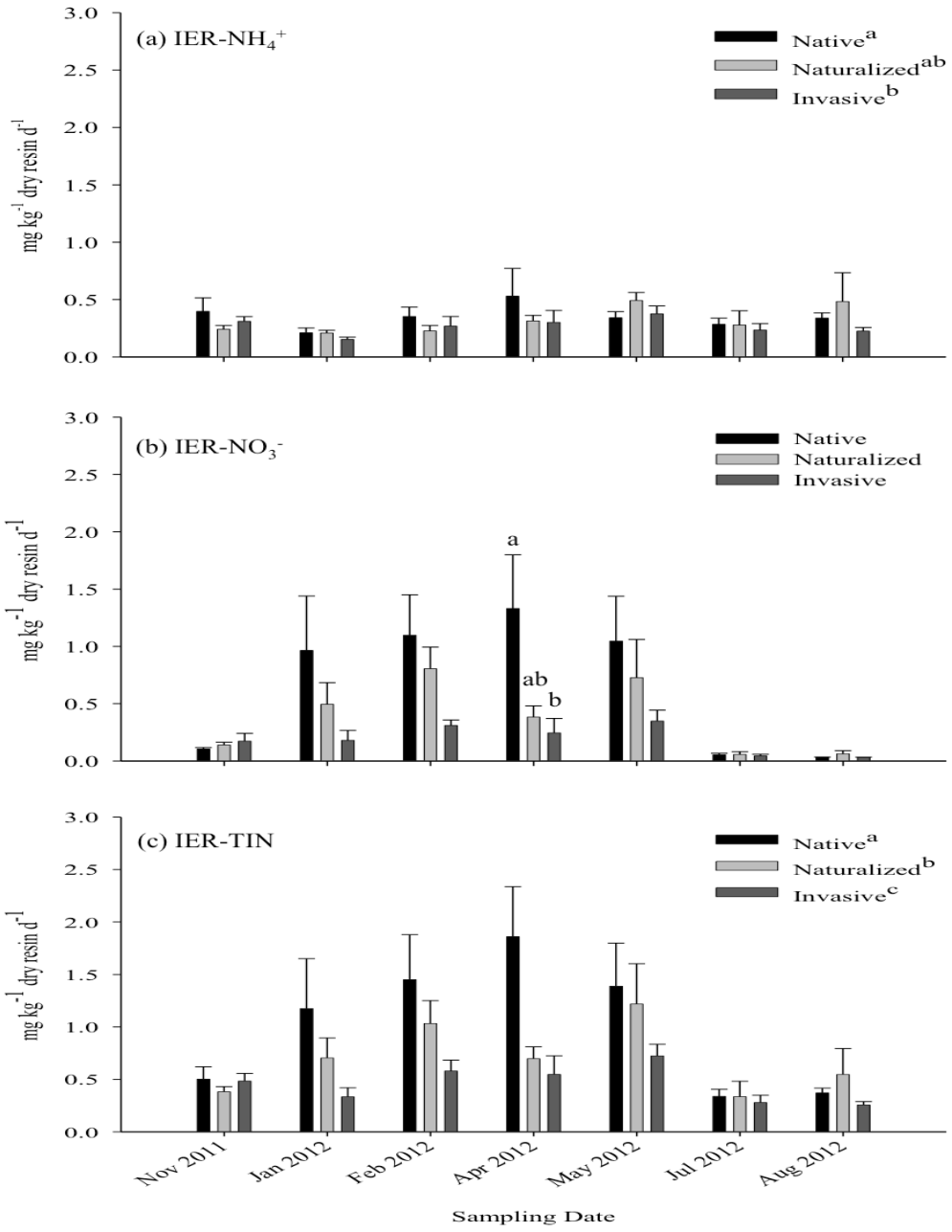

Figure 2-2 The response of ion-exchange resin (a) NH_4^+, (b) NO_3^-, and (c) total inorganic N to different plant communities. Bars ± 1 standard error of the mean (untransformed; n = 12). Different letters denote significant differences by plant community type (determined by a Tukey post-hoc test [$p < 0.05$]). IER-NO_3^- showed a significant treatment by date interaction, with values affected by community type in April.

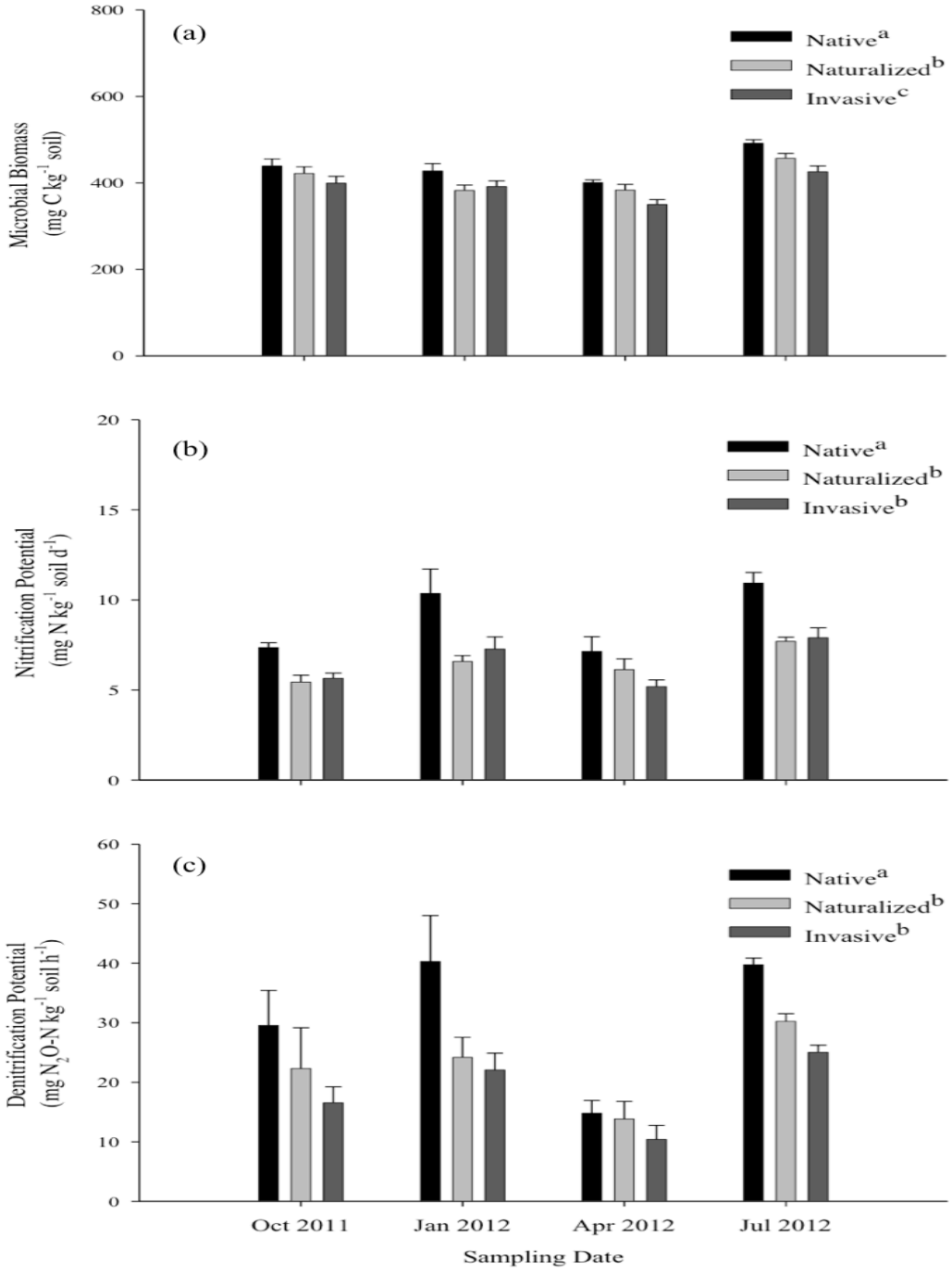

Figure 2-3 Changes in (a) microbial biomass, (b) nitrification potential and (c) denitrification potential as influenced by plant community type over time. Bars ± 1 standard error of the mean (n = 8). Different letters denote significant differences by plant community type (determined by a Tukey post-hoc test [$p < 0.05$]). Interactions between plant community type and date were nonsignificant.

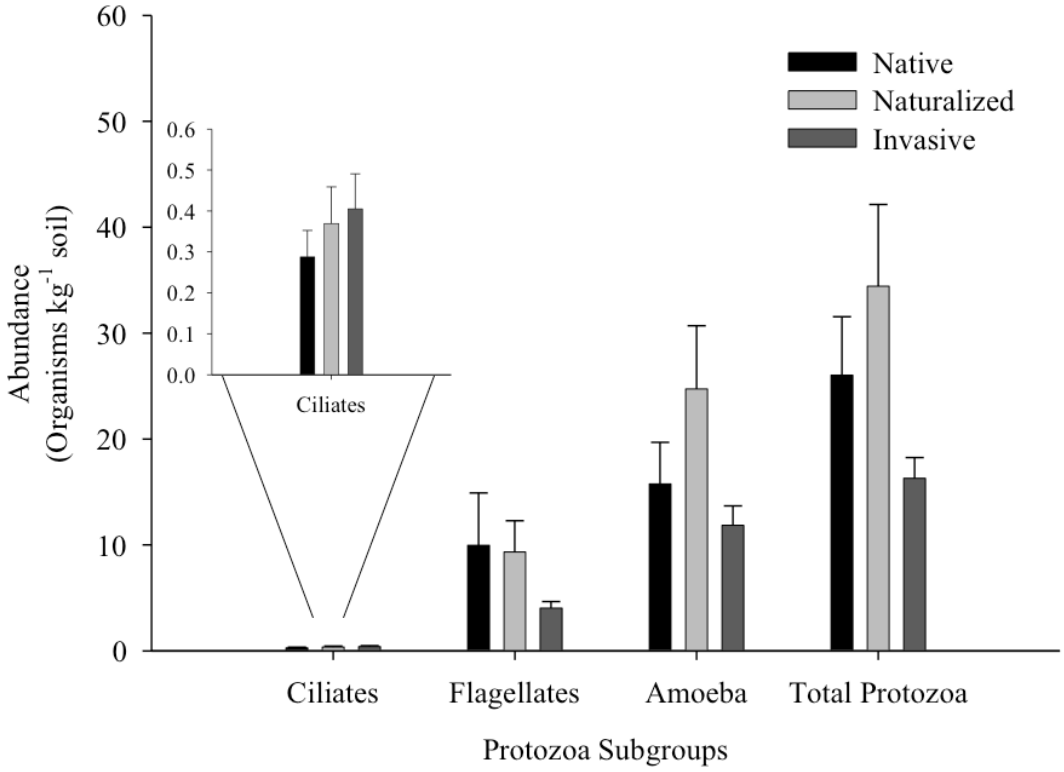

Figure 2-4 Plant community effects on the abundance of ciliates, flagellates, amoeba, and total protozoa in April 2011. Values are means ± 1 SE (n = 10). Different letters denote significant differences by plant community type (determined by a Tukey post-hoc test [$p < 0.05$]).

3 Nitrogen fertilization and defoliation influence soil nitrogen cycling but not the abundance of ammonia oxidizing microorganisms in an invaded grassland community

3.1 Abstract

The ecology of California has seen marked transformations since western colonization. In particular, invasive annual grasses from Mediterranean Europe have replaced many of the native grasslands once dominated by perennial bunchgrasses and annual forbs. Most of these grasslands are grazed extensively by livestock and receive elevated rates of nutrient input through atmospheric deposition. These environmental changes simultaneously affect the landscape; however, little is known about their potential interactive effects on ecosystem structure (e.g., species composition) and function (e.g., nutrient cycling). Our study used experimental grassland plots to measure (1) how soil N cycling responds to changes in plant community composition with invasion by exotic annual grasses and (2) how N additions and defoliation (to simulate one component of cattle grazing: aboveground vegetation removal) affect N cycling individually and in combination. We collected samples seasonally across 2 years and measured instantaneous and accumulated NH_4^+ and NO_3^- concentrations (using KCl soil extractions and ion-exchange resin bags, respectively), potential rates of nitrification and denitrification, and total microbial biomass. In order to gain a mechanistic understanding of changes in nitrification—a key process in the N cycle—we also quantified bacterial and archaeal *amoA* gene abundance, which regulates the first and rate-limiting step of nitrification. We found no evidence that suggests N cycling responds in a non-additive way to the interaction of N fertilization and defoliation. Instead, single factors dominated the response of the system, although some

effects did not persist across years. Invasion, for instance, decreased denitrification potential in the first year and nitrification potential in the second year. Defoliation exacerbated these effects by further reducing nitrification potential and soil NO_3^- availability of invaded grasslands. Fertilization consistently increased nitrification potential and soil NO_3^- concentrations. However, soil NH_4^+ concentrations were rarely affected by treatment, including N fertilization, suggesting that NH_4^+ is either quickly taken up by plants, immobilized by microorganisms, or nitrified to NO_3^-. Surprisingly, changes in nitrification potential were not associated with concurrent shifts in bacterial or archaeal *amoA* gene abundance.

3.2 Introduction

Human-induced global changes, such as climate change (Jones *et al.*, 2009; Bellard *et al.*, 2012), invasion by exotic species (Vitousek *et al.*, 1997; Simberloff, 2011; Stohlgren *et al.*, 2011), increased rates of atmospheric nitrogen (N) deposition (Fenn *et al.*, 2003b; 2010), and land-use change (Asner *et al.*, 2004), are increasingly modifying terrestrial ecosystems worldwide. It is well established that individual global change factors can alter the above- and below-ground structure and functioning of terrestrial ecosystems, such as the composition of plant (Clark & Tilman, 2008; Bobbink *et al.*, 2010), and soil microbial communities (Treseder, 2008; He *et al.*, 2010; Blankinship *et al.*, 2011), and net primary production (NPP; Nemani *et al.*, 2003; Heisler-White *et al.*, 2008). However, much less is known about how interactions among different forms of global change will affect terrestrial ecosystems, despite the fact that individual changes rarely occur in isolation.

In an effort to understand interactive effects, a growing number of studies have manipulated two or more global change factors simultaneously (e.g., Majdi & Öhrvik, 2004; Henry *et al.*, 2005; Niboyet *et al.*, 2011b; Dieleman *et al.*, 2012). Of these, many have focused on the response of soil N cycling (Horz *et al.*, 2004; Barnard *et al.*, 2006; Niboyet *et al.*, 2010; Dijkstra *et al.*, 2010; Niboyet *et al.*, 2011a; Brown *et al.*, 2012; Mueller *et al.*, 2013), given that N limits plant growth in most terrestrial systems (Vitousek & Howarth, 1991; LeBauer & Treseder, 2008). Results of these multifactor global change experiments show wide variation in responses, from non-interactive, to synergistic, to antagonistic. For example, Niboyet *et al.*, (2011c) concluded that global change effects on soil N cycling are primarily additive and as such can be predicted by combining single factor studies. In contrast, Brown *et al.*, (2012) found the presence of a dampening effect with increased multiple global change factors on soil N_2O fluxes, suggesting that combining the effects of single factor studies may overestimate the change in N_2O production. The variation in observed results displays the complexity of interactive effects, and highlights the need for continued research.

In order to obtain a holistic understanding of ecosystem response to global change, it is critical to discern the underlying mechanisms controlling said responses. For terrestrial N cycling, that requires measuring the abundance, composition, and activity of the soil

microbial community. Microorganisms drive terrestrial nutrient cycling (Conrad, 1996; Hayatsu *et al.*, 2008; Schimel & Schaeffer, 2012; Offre *et al.*, 2012) and alterations in ecosystem-level processes are likely linked to microbial abundance and community composition, especially for specialized processes such as nitrification (Schimel, 1995). The accumulating evidence for shifts in microbial community structure with single (Kourtev *et al.*, 2002; Batten *et al.*, 2006; He *et al.*, 2010; Ramirez *et al.*, 2012) and multiple global change factors (Olsrud *et al.*, 2004; Horz *et al.*, 2004; Castro *et al.*, 2010; Gray *et al.*, 2011; Gutknecht *et al.*, 2012; Steinweg *et al.*, 2013) is not surprising, given that microbial communities are sensitive to abiotic (e.g., soil moisture and temperature) and biotic (e.g., plant community composition) variables that are affected by global change. However, there is a need for additional studies that simultaneously measure N cycling processes and microbial community composition within the context of multiple global changes.

Our study addressed two overarching questions: how do invasive plants alter N cycling processes in a California grassland and how do defoliation ('clipping', to simulate one component of livestock grazing) and N fertilization (to simulate elevated N deposition) interact to affect N cycling of invaded grasslands? Barb goatgrass (*Aegilops triuncialis*) and Medusahead (*Elymus caput-medusae*), the two invasive plants utilized in this study, both have relatively low litter quality (Bovey *et al.*, 1961; Swenson *et al.*, 1964; Eviner, 2004; Drenovsky & Batten, 2007), which may reduce decomposition rates (Bovey *et al.*, 1961; Drenovsky & Batten, 2007) and subsequent N cycling compared to native plant communities (as was demonstrated previously between monocultures at this site). As such, we hypothesized that invaded plant communities (mixtures of natives, naturalized, and invasive species) would exhibit decelerated rates of N cycling compared to native-only communities (hypothesis 1). Defoliation can affect N cycling through a number of mechanisms, including alterations to aboveground litter inputs, root growth, C exudation, and soil microclimate (Bardgett *et al.*, 1998); we hypothesized that clipping would slow N cycling, potentially as a result of reducing aboveground litter inputs into the soil system, or increasing plant uptake and microbial immobilization of N (hypothesis 2). The addition of N fertilizer (as NH_4NO_3) can affect soil N cycling through multiple direct and indirect effects; enhanced N supply can directly affect NH_4^+ and NO_3^- pool sizes, or indirectly affect N availability through changes in plant community composition, plant growth, and decomposition rates (Carreiro *et al.*, 2000; Fenn *et al.*, 2003a; Knorr *et al.*, 2005; Manning *et al.*, 2006). We hypothesized that the direct effects of increasing N availability would predominate at our site, resulting in increased rates of N cycling (hypothesis 3). Finally, we hypothesized that N fertilization and clipping would interact in non-additive antagonistic ways (hypothesis 4).

In conjunction with N cycling measurements, we quantified the abundance of ammonia oxidizing bacteria (AOB) and archaea (AOA) based on ammonia monooxygenase (*amoA*) genes. Ammonia oxidizing bacteria and AOA perform the first and rate-limiting step of nitrification (i.e., the conversion of ammonium to nitrite), and have shown sensitivity to changes in plant composition (Hawkes *et al.*, 2005; Boyle-Yarwood *et al.*,

2008) and substrate (NH_4^+) availability (Verhamme *et al.*, 2011; Shen *et al.*, 2011; Wessén & Hallin, 2011). In particular, AOB tend to prefer high NH_4^+ conditions while AOA tend to do better when NH_4^+ availability is low (Verhamme *et al.*, 2011); consequently we expected that soils with elevated N availability would be associated with higher AOB/AOA ratios.

To address our hypotheses, we utilized a well-replicated field experiment comprised of five treatments that had been established three (N fertilization and clipping treatments) to five years (plant communities) in an abandoned agricultural field of California. Samples were collected seasonally over two years, allowing us to determine whether the effects of global change factors are dependent upon time in a Mediterranean climate characterized by large seasonal variations in temperature and precipitation.

3.3 Methods

3.3.1 Site Description & Experimental Design

This study was conducted at an experimental site located in Davis, California (38°32'45.52"N, 121°47'05.37"W). Soils are dominated by the Reiff series (75%, coarse-loamy, mixed, superactive, nonacid, thermic Mollic Xerofluvents) and to a lesser extent the Brentwood soil series (25%, fine, smectitic, thermic Typic Haploxerepts) with a 0-2% slope (USDA Web Soil Survey). The site experiences a Mediterranean climate with a mean annual temperature (MAT) of 15.7°C and mean annual precipitation (MAP) of 485 mm (Figure 3-1).

Treatments are organized in a randomized complete block design and replicated in a factorial manner. Plots are 1.5 m^2 and are separated by a 1-m buffer zone. Five treatments were utilized in this study (Native, Invaded, Fertilized, Clipped, Fertilized + Clipped ['Both']). The native treatment contained plants native to California grasslands (*Bromus carinatus, Elymus glaucus, Elymus triticoides, Ascispon americanus, Lupinus bicolor, Stipa pulchra, Poa secunda*, and *Festuca microstachys)*. The invaded treatment consisted of native, exotic, and invasive plants (*Bromus carinatus, Elymus glaucus, Elymus triticoides, Ascispon americanus, Lupinus bicolor, Stipa pulchra, Poa secunda*, and *Festuca microstachys, Avena fatua, Bromus hordeaceus, Festuca perennis*, and *Trifolium subterraneum, Aegilops triuncialis* and *Elymus caput-medusae*). Plant communities were established in 2006 by adding 139 g of seed to each 1.5 m^2 plot, with an equal proportion of each species in the native treatment. The invaded treatment was seeded with an equal proportion (33%) of natives, naturalized, and invasive species; within each group there was an equal proportion of the component species. The site was irrigated prior to seeding in order to exhaust the existing seed bank; plants that subsequently germinated were treated with glyphosate. Non-planted species are weeded annually.

Fertilized and clipped treatments were initiated in 2008 on invaded plant communities only, because this mixed community type is a more realistic representation of grassland

composition today. For fertilized treatments, NH_4NO_3 fertilizer is applied two to three times a year, totaling an annual rate of 4.5 g NH_4NO_3-N m^{-2} yr^{-1}. Clipped treatments are maintained by clipping and removing aboveground vegetation at a height of 5 cm each spring. In 2011-12, the first year of our study, fertilizer was applied on November 29, 2011 and February 16, 2012. Aboveground vegetation was clipped between April 17 and April 27, 2012. In the second year (2012-13), fertilizer was applied on December 4, 2012, February 20, 2013, and March 26, 2013. Plots were clipped between January 11 – 16, 2013 and again on May 2, 2013.

3.3.2 Sample Collection

In order to account for the strong seasonal variation at our site that is typical of Mediterranean-type ecosystems, we sampled soil four times per year (eight dates total): once in fall (October/November), winter (January), spring (April), and summer (July) seasons. Within each date, five randomly selected mineral soil cores (2.5 cm diameter x 15 cm deep) were composited per plot (n = 8). Composite field-moist samples were sieved (2 mm) and either immediately frozen at -20 °C for molecular analysis, or stored at 4 °C for all other analyses (maximum 3 weeks).

Ion-exchange resin (IER) bags were used to measure the accumulation of NH_4^+ and NO_3^- over time (Binkley & Matson, 1983; Binkley, 1984) and were buried at a depth of 7.5 cm (1 bag/plot) and incubated for 4-7 weeks (n = 12). At the end of each incubation period, resin bags were removed and replaced with new ones using the same hole. The first bags were buried in October 2011 and the last bags were removed in August 2013, totaling 15 consecutive incubations.

Volumetric water content (VWC) was measured at each sampling date using a Time-Domain Reflectometer (TDR; MiniTrase, Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Fifteen-cm long TDR probes were inserted vertically into the soil in October 2011 and 30-cm long probes were installed adjacent to the 15-cm probes in January 2012 (n = 6/depth). This design allowed measurement of VWC within the 0-15 cm and 15-30 cm soil layers.

3.3.3 Soil Characteristics

Soil pH was measured on a 1:2 soil to 0.01 M $CaCl_2$ suspension, which was allowed to equilibrate for 30 minutes prior to analysis using a pH electrode (Orion DUAL STAR meter, Thermo Scientific, Waltham, MA, USA). In 2011-12, soil temperature was measured at every sampling date using a traceable metal thermometer (Control Company, Friendswood, TX, USA) inserted 7.5 cm into the soil. In December 2012, we began measuring temperature using HOBO dataloggers (Onset Corporation, Cape Cod, MA, USA) placed at 7.5 cm depth (1 sensor/plot, n = 8) to get a continuous, time-integrated measurement of soil temperature. For the purpose of this dissertation, the temperature data collected from the HOBO sensors was distilled down to three point measurements

that approximated the time of soil sampling (12:00 PM on 1/9/2014, 4/9/2014, and 7/9/2014). Gravimetric water content (GWC) was determined by drying field moist soil at 105 °C to a constant mass. The dried soil was subsequently ground and used to analyze total carbon (C) and N on an Elemental Combustion System (Costech Analytical Technologies Inc., Valencia, CA, USA). Because total C and N were not expected to vary greatly within one year (Binkley & Hart, 1989), samples collected from the same year were composited over time prior to analysis.

3.3.4 Nitrogen Availability

Instantaneous measurements of soil NH_4^+ and NO_3^- pools were conducted by extracting 15 g field-moist soil with 100 mL 2 M KCl. Samples were shaken for 1 hour on a reciprocating shaker, filtered using pre-leached Whatman No. 1 filter paper, and stored at -20 °C until analysis on a Lachat AE Flow Injection Autoanalyzer (Lachat Instruments, Inc., Milwaukee, WI, USA).

In-situ rates of soil NH_4^+ and NO_3^- accumulation over time were approximated using IER bags (Binkley & Matson, 1983; Binkley, 1984). Briefly, 15 mL of cation and anion resin beads (J.T. Baker Mixed Bed Exchange Resin, IONAC NM-60 H^+/OH^- Form, Type 1, 16-50 Mesh) were measured into nylon stockings, tied shut, and placed in the field (see *Sample Collection* for field incubation details). After removal from the field, resin bags were air dried and placed into 125 mL flasks with 50 mL of 2M KCl. Samples were shaken uncovered for 1 hour on an orbital shaker (180 rpm), filtered using pre-leached Whatman No. 1 filter paper, and stored at -20 °C until analysis on a Lachat AE Flow Injection Autoanalyzer.

3.3.5 Potential Assays and Microbial Biomass

Potential rates of nitrification were estimated using the shaken soil-slurry method (Hart *et al.*, 1994). Specifically, 15 g of field-moist soil was amended with 100 mL NH_4^+ and PO_4^{3-} solution (1.5 mM of NH_4^+ and 1 mM of PO_4^{3-}, pH = 7.2) in a 250 mL flask and capped with a rubber stopper (containing a hole to allow gas exchange but minimize water loss). Flasks were placed on an orbital shaker (180 rpm) for 24 h. At 2, 4, 22, and 24 h, 10 mL of suspension was removed from each flask and centrifuged at 8,000 x g for 8 minutes. Five mL of supernatant was removed from the centrifuged sample, placed into a disposable polypropylene tube, capped, and stored at -20°C until analysis for NO_3^- on a Lachat AE Flow Injection Autoanalyzer.

Potential rates of denitrification were measured using the protocol described by Smith & Tiedje, (1979). Briefly, 50 g of field-moist soil was combined with NO_3^- and labile C (0.1 mg $N\text{-}NO_3^-$ g^{-1} soil, 1 mg C-glucose g^{-1} soil, and 1 mg C-glutamic acid g $soil^{-1}$) in a 250 mL flask, which was subsequently sealed tight with a rubber stopper fitted with a rubber septum. Flasks were alternately evacuated (3 minutes) and flushed with N_2 (1 minute) three times to create anaerobic conditions and then injected with 20 mL of acetylene in

order to inhibit the reduction of N_2O to N_2. The soils were incubated for 90 minutes on an orbital shaker (180 rpm) and sampled at 30 and 90 minutes by removing 15 mL of the headspace. Gas samples were stored in evacuated Exetainer® until analysis for N_2O production on a Shimadzu GC-2014 electron capture detector (Shimadzu Corporation, Columbia, MD, USA).

Microbial biomass was determined using substrate-induced respiration (SIR; West & Sparling, 1986), a method that estimates the abundance of active and glucose-responsive microorganisms in the soil (Wardle & Ghani, 1995). Fifteen g of field-moist soil and 30 mL of a glucose solution (30 mg glucose ml^{-1} H_2O) were added to a 250 mL flask. Flasks were sealed tight with a rubber stopper fitted with a rubber septum and placed on an orbital shaker (180 rpm) for 2.5 h. Fifteen mL of the headspace was sampled at 0.5, 1.5, and 2.5 h; gas samples were stored in evacuated Exetainer® until analysis for CO_2 production on a Shimadzu GC-2014 thermal conductivity detector.

3.3.6 Quantification of amoA gene abundance

Between 0.20-0.30 g of soil from each sample was extracted using a MO BIO PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's instructions. Extracted DNA was quantified using a Quant-iT PicoGreen ds DNA Assay Kit (Life Technologies, Carlsbad, CA, USA) and diluted to 1 ng μL^{-1} DNA for subsequent analyses. Quantitative polymerase chain reaction (qPCR) followed Beman *et al.*, (2012). Bacterial and archaeal *amoA* genes were quantified using the bacterial AmoA1F/amoA2R (Rotthauwe *et al.*, 1997) and archaeal Arch-amoAF/Arch-amoAR (Francis *et al.*, 2005) primer sets; both amplify a broad range *amoA* sequence types from soils (Hawkes *et al.*, 2005; Di *et al.*, 2009; Stopnisek *et al.*, 2010; Zhou *et al.*, 2014). Twenty-five µL PCR reactions for archaeal *amoA* contained SYBR Premix F (Epicentre BioTechnologies, Madison, WI, USA), 2 mM MgCl2, 1.25 units AmpliTaq polymerase (Life Technologies Corporation, Carlsbad, CA, USA), 40 ng μL^{-1} BSA (Life Technologies Corporation, Carlsbad, CA, USA), 0.4 µM forward and reverse primer, and 1 ng μL^{-1} DNA. Reaction chemistries for bacterial and archaeal *amoA* were identical, except the reaction for bacteria did not include $MgCl_2$.

Serial dilutions (10^7 to 10^2 copies) of the archaeal and bacterial *amoA* standards were used to quantify *amoA*. Standards were synthesized at Life Technologies (archaeal *amoA*) or Blue Heron Biotechnologies (bacterial *amoA*) based on previously cloned and sequenced *amoA* genes (archaeal *amoA* clone GOC-G-60-9; GenBank accession no. EU340472; bacterial *amoA* clone HB_A_0206_GO1; GenBank accession no. EU155190). Cycling conditions for AOB were: 95°C (5 min); 40 × 95°C (45 s), 56°C (30 s), 72°C (60 s); 81°C (7 s); dissociation curve. Cycling conditions for AOA were: 95°C (4 min); 40 × 95°C (30 s), 53°C (45 s), 72°C (60 s); dissociation curve. Efficiencies for AOB and AOA *amoA* amplification ranged from 0.87 to 0.94 (R^2 0.93 – 1.0) and 0.86 to 1.02 (R^2 0.91-1.0), respectively.

3.3.7 Statistical Analysis

Two-way repeated measures analysis of variance (ANOVA) was used to analyze all measured variables as a function of treatment and time ($\alpha = 0.05$). In some cases, data was transformed to fit the assumption of normality. To complement the analysis of variance, which tests for treatment interactions that are not additive, we compared observed interactive effects to expected interactive effects. This method provides information on the magnitude and direction of interactions regardless of statistical significance. We used the same method of analysis as Brown *et al.*, (2012), and focused on the additive model. Observed and expected values were calculated using paired samples from within each block, resulting in eight replicates per calculation. The effect size of each individual factor (N fertilization and clipping) was calculated as:

$$X_N = T - C$$

$$X_{Cl} = T - C$$

where T is the single treatment value and C is the control value. Observed combined effects of both factors (N fertilization x clipping) were determined as:

$$X_{observed} = NCl - C$$

where NCl is the observed value for combined treatments and C is the control value. Expected combined effects of both factors based on an additive model were determined as:

$$X_{expected} = X_N + X_{Cl}$$

The interaction term (I)X was calculated by subtracting the expected interactive effect from the observed interactive effect:

$$I(X) = X_{observed} - X_{expected}$$

Interaction terms are presented as mean ± 95% confidence intervals (CI); if the CI does not overlap zero, then the observed interaction deviated from an additive model (above zero, the interaction is synergistic; below zero, the interaction is antagonistic).

Potential rates of nitrification and denitrification were correlated with bacterial and archaeal *amoA* gene abundances and soil properties (e.g., NH_4^+ and NO_3^- concentrations) using Spearman's rank correlation coefficients.

3.4 Results

3.4.1 Effects of Invasion

Invaded treatments showed significant differences compared to native treatments in N concentrations, potential rates of nitrification and denitrification, and microbial biomass. Specifically, in 2011-12, invaded treatments had lower IER-NO_3^- values (Table 3-1), reduced rates of denitrification potential in January, and lower soil microbial biomass across time (-10%, $P < 0.001$, Fig. 1a). In addition, invaded treatments had lower VWC from 0-15 cm (marginally significant, $P = 0.06$) and higher VWC from 15-30 cm depth ($P < 0.01$). All other measured variables were statistically similar between these treatments in 2011-12. In 2012-13, IER-NO_3^- was higher in invaded treatments ($P < 0.05$; Table 3-1), which is in contrast to the pattern observed in the previous year. Potential nitrification ($P < 0.001$), microbial biomass (-8%, $P < 0.001$), total C (-3%, $P < 0.001$) and total N (-3%, $P < 0.001$), however, were lower in invaded treatments. Volumetric water content from 15-30 cm depth remained elevated in invaded treatments ($P < 0.01$). In 2012-13, temperature was significantly reduced in native compared to invaded soils ($P < 0.001$); the appearance of community effects on temperature during the second year may have to due with the use of a more sensitive method of measurement (HOBO dataloggers). No other measured variables were significantly different between these treatments in 2012-13

3.4.2 Effects of N fertilization, clipping, and N fertilization x clipping

Nitrogen fertilization increased soil NO_3^- pools, as demonstrated by both the instantaneous KCl extractable soil NO_3^- and IER-NO_3^- concentrations. In 2011-12, soil NO_3^- concentrations were unaffected by fertilization except in January ($P < 0.001$; Table 3-2, Figure 3-3); however, IER-NO_3^- showed large responses to fertilization throughout most of the 2011-12 growing season (November – April; N fertilization, +443%, $P < 0.001$; Table 3-2, Figure 3-4). Despite the addition of fertilizer as NH_4NO_3, neither soil NH_4^+ nor IER-NH_4^+ concentrations were significantly affected in fertilized plots; however, IER-NH_4^+ and soil NO_3^- showed a decrease with clipping. In 2012-13, NO_3^- availability remained elevated in fertilized treatments, with IER-NO_3^- values again showing a more dramatic response (+504%, $P < 0.001$; Table 3-2) than soil NO_3^- concentrations. However, the dramatic effects of fertilization on IER-NO_3^- values were not apparent or statistically significant in October and February. Clipping increased soil NO_3^- concentrations but reduced IER-NO_3^- values. Similar to 2011-12, neither soil NH_4^+ nor IER-NH_4^+ concentrations were affected by treatment in 2012-13. None of the N availability indices showed a significant interactive response to N fertilization and clipping using repeated measures two-way ANOVA (Table 3-2) and comparison of observed and expected interactive effects (Figure 3-5).

Similar to N availability, fertilization and clipping had large independent effects on N potentials and soil microbial biomass. In 2011-12, N fertilization significantly increased

nitrification potential (Table 3-2) and microbial biomass (Figure 3-6). In contrast, nitrification potential was reduced in clipped treatments, although in April this reduction was only marginally significant. In 2012-13, nitrification potential remained elevated in fertilized plots. Fertilization reduced denitrification potential during January of this year. Similar to 2011-12, clipped treatments showed a significant reduction in nitrification potential in 2012-13. Clipping also increased denitrification potential (Table 3-2) and microbial biomass compared to unclipped treatments (Figure 3-5). Similar to N availability indices, interactions between N fertilization and clipping did not deviate from an additive expectation for either year. This was demonstrated both with the repeated measures two-way ANOVA and by comparing observed to expected interactive effects Figure 3-5. In contrast to effects on potential rates of nitrification, *amoA* gene copy numbers for AOB and AOA were not significantly affected by N fertilization or clipping in 2012-13 Figure 3-7.

Both fertilization and clipping had moderate effects on other ancillary soil properties (Table 3-3). In 2011-12, fertilization slightly reduced soil pH (-0.6%, $P < 0.05$) and VWC at 0-15 cm depth (-2%, $P = 0.09$) and more strongly reduced total N (-6%, $P < 0.05$) and C (-13%, $P = 0.06$), although the effects on total C and VWC were only marginally significant. Clipping slightly reduced soil pH (-0.5%, $P = 0.05$) and induced a stronger, but marginal, reduction on VWC at 15-30 cm depth (-9%, $P = 0.06$). In contrast, clipping increased VWC at 0-15 cm depth (+9%, $P =0.06$), but again this effect was marginally significant. Similar to 2011-12, fertilization in 2012-13 slightly reduced soil pH (-0.2%, $P = 0.06$) and 0-15 cm VWC (-2%, $P < 0.05$). In July of 2012-13, fertilized treatments had marginally significant higher GWC (+8%, $P = 0.08$) than plots that were not fertilized. Total C and N showed no significant variation with treatment in the second year of the study. Volumetric (0-15 cm) and GWC in 2012-13 both exhibited a marginally significant interactive response to N fertilization and clipping ($P = 0.09$ and $P = 0.08$, respectively). Temperature in 2012-13 showed a significant treatment by date interaction. No significant differences occurred in January; however, in April, clipped treatments were warmer than unclipped and fertilized treatments. In July, the invaded control and fertilized treatment were cooler than plots that were clipped (both the clipped only and fertilized + clipped treatments).

3.4.3 Correlations among response variables

Nitrification potential showed contrasting relationships with AOB and AOA *amoA* gene abundance, although these correlations were marginal and did not remain consistent across time (Table 3-4). In particular, nitrification potential was positively correlated with AOB abundance in November and July ($P < 0.1$) and negatively correlated with AOA abundance in July ($P < 0.1$). Ammonia oxidizing bacterial abundance was negatively correlated with soil temperature in January. Archaeal abundance, however, was negatively correlated with most N availability metrics, but positively correlated with soil temperature, in July. AOA was also positively correlated with soil moisture in November and April.

Potential rates of nitrification and denitrification were correlated with a number of other soil characteristics measured in this study (Table 3-5). In 2011-12 and 2012-13, both potentials tended to show a strong positive correlation with soil microbial biomass. Nitrification potential was also positively correlated with soil NO_3^- concentrations across most dates. IER-NH_4^+ and IER-NO_3^- values rarely correlated with potential rates; the only significant relationships occurred in July when nitrification was positively correlated with IER-NH_4^+ and denitrification was positively correlated with IER-NO_3^-. Soil moisture and pH showed both positive and negative correlations with nitrification and denitrification potentials; however, these relationships were intermittent.

3.5 Discussion

Our study utilized a well-replicated field experiment, and a combination of in situ measurements, potential assays, and molecular tools to address two questions of importance for Mediterranean grasslands in an era of global change: (1) how do invasive plants influence soil N dynamics and (2) how do N dynamics in invaded grasslands respond to the interaction of N fertilization (simulated N deposition) and defoliation (simulated grazing)? We demonstrate that soil N cycling and availability, but not the abundances of ammonia oxidizing microorganisms, are sensitive to each global change factor. Importantly, we also show that N fertilization and defoliation interact in an additive way.

3.5.1 Response of soil N cycling to exotic plant invasion

Consistent with our first hypothesis, invaded plant communities exhibited decelerated rates of potential nitrification and denitrification compared to native communities. These effects, however, did not persist across years and significant changes in denitrification potential (in 2011-12) were decoupled from changes in nitrification potential (in 2012-13). This is likely because these processes are regulated by different factors; for instance, organic matter (C) and NO_3^- are essential for denitrification (Wallenstein *et al.*, 2006), whereas O_2 and NH_4^+ availability are requirements for nitrification (Chapin *et al.*, 2012). Thus, the transient nature of plant community effects on potential rates may have been a result of changes in relative abundances of plant species within community type (Eviner *et al*., unpublished), or inter-annual fluctuations in soil moisture availability (Figure 3-2) that may have differentially affected the potential for the soil to nitrify or denitrify.

Reductions in soil total C and N with invasion were not apparent in 2011-12, but became significant in 2012-13, suggesting that *A. triuncialis and E. caput-medusae* were beginning to influence total soil C and N pools. There are examples where the presence of invasive plant populations for over 40 years at a site has not resulted in changes to C and N storage capacity of the soil (Svejcar & Sheley, 2001). That *A. triuncialis and E. caput-medusae* affected total C and N indicates that the lower litter quality and reduced decomposability (Bovey *et al.*, 1961; Drenovsky & Batten, 2007) of these species has the

potential to transform "slow-changing" soil variables within just a few years of establishment.

Despite the fact that total soil N and nitrification potential were reduced with invasion, ammonia-oxidizing bacteria and AOA *amoA* gene abundances were unaffected by plant community type. Effects of invasion are thought to be dependent upon the functional distinctiveness of invasive species compared to resident species (Vitousek *et al.*, 1987; Levine *et al.*, 2003; González-Muñoz *et al.*, 2012). As such, it is possible that key traits (e.g., litter quality and N uptake patterns) of *A. triuncialis and E. caput-medusae* are not different enough to induce ecologically significant changes in soil variables (e.g., NH_4^+ availability, soil moisture) that control the abundance of ammonia oxidizing microorganisms, at least as measured by qPCR of *amoA* genes.

It is more likely, however, that by comparing native plant communities with mixed communities comprised of native, naturalized, and invasive plants, the effects of *A. triuncialis and E. caput-medusae* may have been diluted. Indeed, previous work in an annual grassland showed that AOB gene abundance was significantly increased in soils associated with exotic grasses, and that this increase translated into high gross rates of nitrification (Hawkes *et al.*, 2005). However, these effects were only significant when comparing exotic and native species monocultures; when comparing native communities to mixed communities of native and exotics, changes in AOB abundance and nitrification were no longer significant. Similarly, in a study comparing the effects of exotic annual grass abundance on N cycling in a California grassland, the greatest differences in soil inorganic N, N cycling rates, and microbial biomass occurred between plots dominated by exotic annuals and plots dominated by perennials (Corbin & D'Antonio, 2011). In the same way, it is possible that we would have detected changes in *amoA* gene abundances if monocultures of *A. triuncialis* and *E. caput-medusae* were compared to monocultures of natives. Future studies assessing *amoA* gene copy number in plant communities with varying degrees of invasive species abundance will be important to determine if a there is a threshold after which the growth of ammonia oxidizing microorganisms is altered.

Overall, our results demonstrate that, even when not in monoculture, the presence of *A. triuncialis* and *E. caput-medusae* can reduce soil C and N and the potential for the soil to nitrify and denitrify. Changes in potential rates were associated with lower active (total) microbial biomass, but, surprisingly, nitrification potential was unrelated to changes in AOB or AOA abundance. This suggests that activity or composition rather than abundance of ammonia oxidizing microorganisms may be important in regulating the response of nitrification to exotic plant invasion.

3.5.2 Response of soil N cycling to clipping

Results surrounding our second hypothesis that clipping would reduce N availability were dependent upon year. In 2011-12, IER-NH_4^+, nitrification potential, and soil NO_3^- were reduced with clipping; in 2012-13, nitrification potential and IER-NO_3^- were reduced, but soil NO_3^-, denitrification potential and microbial biomass were increased in

clipped relative to unclipped treatments (soil NO_3^- was increased by 2 %, while IER-NO_3^- was reduced by 11% relative to the control). Although potential rates of nitrification responded to clipping, *amoA* gene abundances remained unaffected.

There are a number of mechanisms by which defoliation can influence soil N cycling, including the reduction of aboveground litter inputs, alterations in root growth and C exudation, and effects on soil microclimate (reviewed by Bardgett *et al.*, 1998). Decomposition of organic matter is a primary pathway by which available N is returned to the soil; as such, annually clipping and removing aboveground vegetation likely had an effect on N availability and microbial-mediated processes. Indeed, the longer-term nature of our study (3 - 4 years) may have increased the relative influence of aboveground litter removal, and could possibly explain why our results contrast with other shorter-term defoliation studies (7 days – 1 year) that reported increases in N availability (Guitian & Bardgett, 2000; Hamilton *et al.*, 2008; Cheng *et al.*, 2011). In addition, accelerated plant uptake (Wallace & Macko, 1993; Bardgett & Wardle, 2003) or microbial immobilization of available N (Magill & Aber, 2000), which can occur in response to defoliation, may partially explain the observed reductions in N availability and potential rates of nitrification in clipped compared to unclipped treatments.

Increases in microbial biomass and denitrification potential during the second year of our study suggest that root exudation of labile C likely influenced the response of soil N cycling to defoliation. Plants can respond to clipping by exuding organic acids, sugars, and amino acids (Bardgett *et al.*, 1998; Hütsch *et al.*, 2002; Hamilton *et al.*, 2008), although this varies by species (Augustine *et al.*, 2011). Thus, root exudation of labile C could explain the observed increases in soil microbial biomass (Holland, 1995; Bardgett *et al.*, 1997) and denitrification potential, given that many microorganisms (including denitrifiers) are heterotrophs. That these increases occurred only during 2012-13, which was a wetter year than 2011-12, intimates that the positive response of microbial processes may be constrained by water availability in this system.

A number of studies have focused on the immediate or short-term responses of soil properties to clipping (Guitian & Bardgett, 2000; Hamilton & Frank, 2001; Hamilton *et al.*, 2008), and others have measured soil N cycling in ungrazed and grazed grasslands (Shariff *et al.*, 1994; Patra *et al.*, 2005; Ingram *et al.*, 2008; Coetsee *et al.*, 2010; He *et al.*, 2011; Hirobe *et al.*, 2013), which cannot necessarily decouple the effects of defoliation, waste excretion, and trampling. Our study subjected experimental grassland communities to annual clipping, and measured soil four times per year (none of which were immediately after clipping). Consequently, we were able to demonstrate that defoliation can have long-term, sustained effects on soil N availability and potential rates of N cycling, which may exacerbate (e.g., N availability and nitrification) or alleviate (e.g., microbial biomass and denitrification) the belowground effects of *A. triuncialis* and *E. caput-medusae* invasion.

3.5.3 Response of soil N cycling to N fertilization

Our third hypothesis, which postulated that N fertilization would accelerate the N cycle, was supported by an increase in potential rates of nitrification across both years. Surprisingly, however, no differences in NH_4^+ concentrations were observed despite fertilization with NH_4NO_3. This suggests that nitrifying microorganisms present in the soil were rapidly converting the added NH_4^+ to NO_3^-, resulting in a substantial increase in NO_3^- availability (443 – 504%). Large accumulations of NO_3^- from deposition of available N could therefore accelerate NO_3^- leaching into groundwater and potentially affect the water quality and ecology of adjacent aquatic ecosystems.

Denitrification potential did not respond to increased NO_3^- availability due to fertilization, suggesting that denitrifying microorganisms may be limited by other factors such as O_2 or C. Indeed, denitrification potential was unaffected by fertilization in 2011-12 and was reduced by fertilization in January 2013. This was surprising given that increases in denitrification potential (Niboyet *et al.*, 2010; 2011b), net denitrification rates (Lu *et al.*, 2010) and soil N_2O fluxes (Ryden, 1983; Velthof & Oenema, 1995; Aronson & Allison, 2012) have been reported with N additions. While fertilized treatments also exhibited reduced pH and volumetric water content, two soil characteristics known to regulate denitrification (Šimek & Cooper, 2002; Wallenstein *et al.*, 2006), the relatively small magnitude of change (-0.2 to -0.6% pH and -2% VWC) makes it unlikely that these variables influenced potential rates of denitrification. Instead, it is possible that decreases in root growth and biomass (Davidson, 1969; Reynolds & D'Antonio, 1996; George & Seith, 1998) or increases in competition with other heterotrophic microorganisms (Barnard *et al.*, 2005) may have reduced C availability for denitrifiers, thus limiting their ability to respond to increases in NO_3^- with fertilization. An increase in total soil microbial biomass during 2011-12 lends evidence towards the second mechanism. Alternatively, potential rates of denitrification may have been reduced because of shifts in the abundance or composition of denitrifying microorganisms. Accumulating evidence suggests that fungi are predominant denitrifiers in soils (Laughlin & Stevens, 2002; Crenshaw *et al.*, 2008; Hayatsu *et al.*, 2008; Marusenko *et al.*, 2013; Mothapo *et al.*, 2013), and although the effects of elevated N supply on fungal biomass and community composition is equivocal (Bittman *et al.*, 2005; de Vries *et al.*, 2006; Treseder, 2008; Porras-Alfaro *et al.*, 2011; Weber *et al.*, 2013), in some scenarios fungi can be negatively affected. As such, changes in fungal community composition, or suppression of fungal biomass, may be partially responsible for the observed reduction in denitrification potential with fertilization.

Despite marked changes in nitrification potential (which approximates the size of the nitrifying community), the abundances of AOB and AOA were not significantly affected by N additions. The lack of overall response of *amoA* gene abundance was unanticipated, given that multiple studies have demonstrated increases in AOB abundance to enhanced N supply (Mendum *et al.*, 1999; Hermansson & Lindgren, 2001; Okano *et al.*, 2004; Di *et al.*, 2010; Shen *et al.*, 2011; Wertz *et al.*, 2012). However, we applied N at lower rates

than many of the studies showing a positive response of AOB, which may explain why our results are incongruent with others; for example, Shen *et al.*, (2011) provide evidence that AOB populations only respond to urea fertilization rates above 8 g N m^{-2} y^{-1}, which is almost double what we applied as NH_4NO_3 in our study. The observed unresponsiveness of AOA abundance to fertilization in our study corresponds with previous work (Di *et al.*, 2010; Stopnisek *et al.*, 2010; Shen *et al.*, 2011; Wertz *et al.*, 2012) and is consistent with the idea that AOA may prefer to utilize N derived from organic matter mineralization rather than inorganic N fertilization (Levičnik-Höfferle *et al.*, 2012). New findings also suggest that AOA growth may be limited by the availability of nutrients such as phosphorus and potassium, thereby restricting their ability to respond to increases in NH_4^+ (Norman & Barrett, 2014).

Nitrification potential and AOB and AOA *amoA* gene abundances were not strongly or consistently related, suggesting that *amoA* gene abundance may not be a good indicator of activity in soils, at least as measured by laboratory potential assays. The neutral to negative relationship between AOA and potential rates of nitrification observed in this study have been found elsewhere (Jia & Conrad, 2009; Bernhard *et al.*, 2010; Di *et al.*, 2010; Wertz *et al.*, 2012; de Gannes *et al.*, 2014) and there are a few likely explanations for why this may be. First, AOA may be inhibited by high amounts of substrate added (Martens-Habbena *et al.*, 2009; Bernhard *et al.*, 2010; Verhamme *et al.*, 2011) or physical perturbation (Röling, 2007; Bernhard *et al.*, 2010) typical of a potential assay. In addition, nitrification rates may be influenced more heavily by the composition, and thus the physiology, of AOA rather than the overall abundance of archaeal *amoA* genes (Röling, 2007; Brankatschk *et al.*, 2010). Lastly, not all AOA may perform ammonia oxidation at a given time (Stahl & la Torre, 2012), which would result in a weak relationship between *amoA* gene abundance and nitrification potential. Given that a strong positive relationship between AOB abundance and nitrification potential was also lacking suggests that the abundance of *amoA* transcripts, rather than *amoA* genes, may be a better indicator of nitrifying activity in these soils.

Overall, our results demonstrate that N cycling of invaded grasslands can be affected by N additions, but that the response of nitrification may be regulated by microbial activity (de Gannes *et al.*, 2014; Graham *et al.*, 2014) that is decoupled from *amoA* gene abundance. Moreover, similar to findings from agricultural soils, dairy pasture soils, and forest soils (Jia & Conrad, 2009; Di *et al.*, 2009; 2010; Wertz *et al.*, 2012), but in contrast to highly acidic soils (Gubry-Rangin *et al.*, 2010; Zhang *et al.*, 2012), our study suggests that ammonia oxidation in Mediterranean-type grasslands may be largely driven by AOB.

3.5.4 Response of soil N cycling to the interaction of N fertilization and clipping

We found no evidence for the interaction of N fertilization and clipping to affect N cycling in a non-additive way. This means that the impact of clipping on soil N cycling was not dependent upon the nutrient status of the soil (fertilized or unfertilized), and that the impact of fertilization did not differ between clipped and unclipped plots. These findings are consistent with previous studies that suggest interactive responses to global change may be rare (Zavaleta *et al.*, 2003; Zhou *et al.*, 2006; Niboyet *et al.*, 2011c). However, they contrast with many other findings (Shaw, 2002; Henry *et al.*, 2005; Barnard *et al.*, 2006; Castro *et al.*, 2010; Blankinship *et al.*, 2010; Dieleman *et al.*, 2012), including those that have found N fertilization and defoliation to produce significant interactive effects on plant root and shoot biomass and compensation patterns (Li & Redmann, 1992; Hawkes & Sullivan, 2001; Ferraro & Oesterheld, 2002). Whether significant non-additive interactions occur may be dependent upon the system, the response variables measured, and the global change factors that were manipulated. Importantly, our results demonstrate that in Mediterranean-type grasslands, the response of soil N cycling to N deposition is not dependent upon the presence of defoliation, and vice-versa.

3.6 Conclusion

The N cycling variables measured in our study responded to the presence of invasive species, N fertilization, and clipping, but not to the interaction of N fertilization and clipping. Invaded soils had decelerated rates of N cycling compared to native soils, and clipping of aboveground vegetation tended to exacerbate low N conditions. In contrast, N fertilization increased nitrification potential and NO_3^- availability, which highlights the possibility for NO_3^- leaching to occur in grassland sites that receive elevated rates of N deposition. Importantly, changes in potential rates of nitrification were not consistently associated with shifts in bacterial and archaeal *amoA* gene abundance, suggesting the likely role of other controlling mechanisms, such as AOB and AOA community composition.

3.7 References

Aronson EL, Allison SD (2012) Meta-analysis of environmental impacts on nitrous oxide release in response to N amendment. *Frontiers in microbiology*, **3**.

Asner GP, Elmore AJ, Olander LP, Martin RE, Harris AT (2004) Grazing systems, ecosystem responses, and global change. *Annual Review of Environment and Resources*, **29**, 261–299.

Augustine DJ, Dijkstra FA, William Hamilton Iii E, Morgan JA (2011) Rhizosphere interactions, carbon allocation, and nitrogen acquisition of two perennial North American grasses in response to defoliation and elevated atmospheric CO2. *Oecologia*, **165**, 755–770.

Bardgett RD, Wardle DA (2003) Herbivore-mediated linkages between aboveground and belowground communities. *Ecology*, **84**, 2258–2268.

Bardgett RD, Leemans DK, Cook R, Hobbs PJ (1997) Seasonality of the soil biota of grazed and ungrazed hill grasslands. *Soil Biology and Biochemistry*, **29**, 1285–1294.

Bardgett RD, Wardle DA, Yeates GW (1998) Linking above-ground and below-ground interactions: How plant responses to foliar herbivory influence soil organisms. *Soil Biology and Biochemistry*, **30**, 1867–1878.

Barnard R, Le Roux X, Hungate BA, Cleland EE, Blankinship JC, Barthes L, Leadley PW (2006) Several components of global change alter nitrifying and denitrifying activities in an annual grassland. *Functional Ecology*, **20**, 557–564.

Barnard R, Leadley PW, Hungate BA (2005) Global change, nitrification, and denitrification: A review. *Global Biogeochemical Cycles*, **19**, 1–13.

Batten KM, Scow KM, Davies KF, Harrison SP (2006) Two Invasive Plants Alter Soil Microbial Community Composition in Serpentine Grasslands. *Biological Invasions*, **8**, 217–230.

Bellard C, Bertelsmeier C, Leadley P, Thuiller W, Courchamp F (2012) Impacts of climate change on the future of biodiversity. *Ecology Letters*, **15**, 365–377.

Beman JM, Popp BN, Alford SE (2012) Quantification of ammonia oxidation rates and ammonia-oxidizing archaea and bacteria at high resolution in the Gulf of California and eastern tropical North Pacific Ocean. *Limnology and Oceanography*, **57**, 711–726.

Bernhard AE, Landry ZC, Blevins A, la Torre de JR, Giblin AE, Stahl DA (2010) Abundance of Ammonia-Oxidizing Archaea and Bacteria along an Estuarine Salinity Gradient in Relation to Potential Nitrification Rates. *Applied and Environmental Microbiology*, **76**, 1285–1289.

Binkley D (1984) Ion Exchange Resin Bags: Factors Affecting Estimates of Nitrogen Availability1. *Soil Science Society of America Journal*, **48**, 1181–1184.

Binkley D, Hart SC (1989) The components of nitrogen availability assessments in forest soils. In: *Advances in Soil Science* pp57–112. New York, Springer-Verlag.

Binkley D, Matson P (1983) Ion exchange resin bag method for assessing forest soil nitrogen availability. *Soil Science Society of America Journal,* **48**, 1181–1184.

Bittman S, Forge TA, Kowalenko CG (2005) Responses of the bacterial and fungal biomass in a grassland soil to multi-year applications of dairy manure slurry and fertilizer. *Soil Biology and Biochemistry*, **37**, 613–623.

Blankinship JC, Brown JR, Dijkstra P, Hungate BA (2010) Effects of interactive global changes on methane uptake in an annual grassland. *Journal of Geophysical Research*, **115**, 1–9.

Blankinship JC, Niklaus PA, Hungate BA (2011) A meta-analysis of responses of soil biota to global change. *Oecologia*, **165**, 553–565.

Bobbink R, Hicks K, Galloway J, et al. (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications*, **20**, 30–59.

Bovey RW, Le Tourneau D, Erickson LC (1961) The chemical composition of medusahead and downy brome. *Weeds*, **9**, 307–311.

Boyle-Yarwood SA, Bottomley PJ, Myrold DD (2008) Community composition of ammonia-oxidizing bacteria and archaea in soils under stands of red alder and Douglas fir in Oregon. *Environmental Microbiology*, **10**, 2956–2965.

Brankatschk R, Töwe S, Kleineidam K, Schloter M, Zeyer J (2010) Abundances and potential activities of nitrogen cycling microbial communities along a chronosequence of a glacier forefield. *The ISME Journal*, **5**, 1025–1037.

Brown JR, Blankinship JC, Niboyet A, et al. (2012) Effects of multiple global change treatments on soil N2O fluxes. *Biogeochemistry*, **109**, 85–100.

Carreiro MM, Sinsabaugh RL, Repert DA, Parkhurst DF (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology*, **81**, 2359–2365.

Castro HF, Classen AT, Austin EE, Norby RJ, Schadt CW (2010) Soil Microbial Community Responses to Multiple Experimental Climate Change Drivers. *Applied and Environmental Microbiology*, **76**, 999–1007.

Chapin FS III, Matson P, Vitousek PM (2012) *Principles of terrestrial ecosystem ecology*. New York, Springer.

Cheng X, Luo Y, Su B, Wan S, Hui D, Zhang Q (2011) Plant carbon substrate supply regulated soil nitrogen dynamics in a tallgrass prairie in the Great Plains, USA: results of a clipping and shading experiment. *Journal of Plant Ecology*, **4**, 228–235.

Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature*, **451**, 712–715.

Coetsee C, Stock WD, Craine JM (2010) Do grazers alter nitrogen dynamics on grazing lawns in a South African savannah? *African Journal of Ecology*, **49**, 62–69.

Conrad R (1996) Soil microorganisms as controllers of atmospheric trace gases (H2, CO, CH4, OCS, N2O, and NO). *Microbiological reviews*, **60**, 609–640.

Corbin JD, D'Antonio CM (2011) Abundance and productivity mediate invader effects on nitrogen dynamics in a California grassland. *Ecosphere*, **2**, art32.

Crenshaw CL, Lauber C, Sinsabaugh RL, Stavely LK (2008) Fungal control of nitrous oxide production in semiarid grassland. *Biogeochemistry*, **87**, 17–27.

Davidson RL (1969) Effects of soil nutrients and moisture on root/shoot ratios in Lolium perenne L. and Trifolium repens L. *Annals of Botany*, **33**, 571–577.

de Gannes V, Eudoxie G, Hickey WJ (2014) Impacts of Edaphic Factors on Communities of Ammonia-Oxidizing Archaea, Ammonia-Oxidizing Bacteria and Nitrification in Tropical Soils. *PLoS ONE,* **9**, e89568.

de Vries FT, Hoffland E, van Eekeren N, Brussaard L, Bloem J (2006) Fungal/bacterial ratios in grasslands with contrasting nitrogen management. *Soil Biology and Biochemistry*, **38**, 2092–2103.

Di HJ, Cameron KC, Shen J-P, Winefield CS, O'Callaghan M, Bowatte S, He J-Z (2010) Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. *FEMS Microbiology Ecology*, **72**, 386–394.

Di HJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S, He JZ (2009) Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nature Geoscience*, **2**, 621–624.

Dieleman WIJ, Vicca S, Dijkstra FA, et al. (2012) Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO 2and temperature. *Global Change Biology*, **18**, 2681–2693.

Dijkstra FA, Blumenthal D, Morgan JA, PENDALL E, Carrillo Y, Follett RF (2010) Contrasting effects of elevated CO2 and warming on nitrogen cycling in a semiarid grassland. *New Phytologist*, **187**, 426–437.

Drenovsky RE, Batten KM (2007) Invasion by Aegilops triuncialis (Barb Goatgrass) Slows Carbon and Nutrient Cycling in a Serpentine Grassland. *Biological Invasions*, **9**, 107–116.

Eviner VT (2004) Plant traits that influence ecosystem processes vary independently among species. *Ecology*, **85**, 2215–2229.

Fenn ME, Allen EB, Weiss SB, et al. (2010) Nitrogen critical loads and management alternatives for N-impacted ecosystems in California. *Journal of Environmental Management*, **91**, 2404–2423.

Fenn ME, Baron JS, Allen EB, et al. (2003a) Ecological effects of nitrogen deposition in the western United States. *BioScience*, **53**, 404–420.

Fenn ME, Haeuber R, Tonnesen GS, et al. (2003b) Nitrogen emissions, deposition, and monitoring in the western United States. *BioScience*, **53**, 391–403.

Ferraro DO, Oesterheld M (2002) Effect of defoliation on grass growth. A quantitative review. *Oikos*, **98**, 125–133.

Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences*, **102**, 14683–14688.

George E, Seith B (1998) Long-term effects of a high nitrogen supply to soil on the growth and nutritional status of young Norway spruce trees. *Environmental Pollution*, **102**, 301–306.

González-Muñoz N, Castro-Díez P, Parker IM (2012) Differences in nitrogen use strategies between native and exotic tree species: predicting impacts on invaded ecosystems. *Plant and Soil*, **363**, 319–329.

Graham EB, Wieder WR, Leff JW, et al. (2014) Do we need to understand microbial communities to predict ecosystem function? A comparison of statistical models of nitrogen cycling processes. *Soil Biology and Biochemistry*, **68**, 279–282.

Gray SB, Classen AT, Kardol P, Yermakov Z, Michael Mille R (2011) Multiple Climate Change Factors Interact to Alter Soil Microbial Community Structure in an Old-Field Ecosystem. *Soil Science Society of America Journal*, **75**, 2217.

Gubry-Rangin C, Nicol GW, Prosser JI (2010) Archaea rather than bacteria control nitrification in two agricultural acidic soils. *FEMS Microbiology Ecology*, **74**, 566–574.

Guitian R, Bardgett RD (2000) Plant and soil microbial responses to defoliation in temperate semi-natural grassland. *Plant and Soil*, **220**, 271–277.

Gutknecht JLM, Field CB, Balser TC (2012) Microbial communities and their responses to simulated global change fluctuate greatly over multiple years. *Global Change Biology*, **18**, 2256–2269.

Hamilton EW III, Frank DA (2001) Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology*, **82**, 2397–2402.

Hamilton EW III, Frank DA, Hinchey PM, Murray TR (2008) Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland. *Soil Biology and Biochemistry*, **40**, 2865–2873.

Hart SC, Stark JM, Davidson EA, Firestone MK (1994) Nitrogen mineralization, immobilization, and nitrification. *Methods of Soil Analysis: Part 2—Microbiological and Biochemical Properties*, 985–1018.

Hawkes CV, Sullivan JJ (2001) The impact of herbivory on plants in different resource conditions: A meta-analysis. *Ecology*, **82**, 2045–2058.

Hawkes CV, Wren IF, Herman DJ, Firestone MK (2005) Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecology Letters*, **8**, 976–985.

Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: Diversity and functions of the microorganisms involved in nitrification and denitrification. *Soil Science and Plant Nutrition*, **54**, 33–45.

He NP, Zhang YH, Yu Q, Chen QS, Pan QM, Zhang GM, Han XG (2011) Grazing intensity impacts soil carbon and nitrogen storage of continental steppe. *Ecosphere*, **2**, art8.

He Z, Xu M, Deng Y, et al. (2010) Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO2. *Ecology Letters*, **13**, 564–575.

Heisler-White JL, Knapp AK, Kelly EF (2008) Increasing precipitation event size increases aboveground net primary productivity in a semi-arid grassland. *Oecologia*, **158**, 129–140.

Henry HA, Juarez JD, Field C, Vitousek PM (2005) Interactive effects of elevated CO2, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. *Global Change Biology*, **11**, 1808–1815.

Hermansson A, Lindgren PE (2001) Quantification of Ammonia-Oxidizing Bacteria in Arable Soil by Real-Time PCR. *Applied and Environmental Microbiology*, **67**, 972–976.

Hirobe M, Kondo J, Enkhbaatar A, et al. (2013) Effects of livestock grazing on the spatial heterogeneity of net soil nitrogen mineralization in three types of Mongolian grasslands. *Journal of Soils and Sediments*, **13**, 1123–1132.

Holland NJ (1995) Effects of above-ground herbivory on soil microbial biomass in conventional and no-tillage agroecosystems. *Applied Soil Ecology*, **2**, 275–279.

Horz H-P, Barbrook A, FIELD CB, Bohannan BJM (2004) Ammonia-oxidizing bacteria respond to multifactorial global change. *Proceedings of the National Academy of Sciences*, **101**, 15136–15141.

Hütsch BW, Augustin J, Merbach W (2002) Plant rhizodeposition — an important source for carbon turnover in soils. *Journal of Plant Nutrition and Soil Science*, **165**, 397.

Ingram LJ, Stahl PD, Schuman GE, et al. (2008) Grazing Impacts on Soil Carbon and Microbial Communities in a Mixed-Grass Ecosystem. *Soil Science Society of America Journal*, **72**, 939.

Jia Z, Conrad R (2009) Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. *Environmental Microbiology*, **11**, 1658–1671.

Jones C, Lowe J, Liddicoat S, Betts R (2009) Committed terrestrial ecosystem changes due to climate change. *Nature Geoscience*, **2**, 1–4.

Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: a meta-analysis. *Ecology*, **86**, 3252–3257.

Kourtev PS, Ehrenfeld JG, Häggblom M (2002) Exotic plant species alter the microbial community structure and function in the soil. *Ecology*, **83**, 3152–3166.

Laughlin RJ, Stevens RJ (2002) Evidence for fungal dominance of denitrification and codenitrification in a grassland soil. *Soil Science Society of America Journal*, **66**, 1540–1548.

LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, **89**, 371–379.

Levičnik-Höfferle Š, Nicol GW, Ausec L, Mandić-Mulec I, Prosser JI (2012) Stimulation of thaumarchaeal ammonia oxidation by ammonia derived from organic nitrogen but not added inorganic nitrogen. *FEMS Microbiology Ecology*, **80**, 114–123.

Levine JM, Vila M, Antonio CMD, Dukes JS, Grigulis K, Lavorel S (2003) Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 775–781.

Li YS, Redmann RE (1992) Growth Responses to Ammonium-N, Nitrate-N and Defoliation in Agropyron dasystachyum from the Canadian Mixed Grassland. *American Midland Naturalist*, **127**, 300–308.

Lu M, Yang Y, Luo Y, et al. (2010) Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. *New Phytologist*, **189**, 1040–1050.

Magill AH, Aber JD (2000) Variation in soil net mineralization rates with dissolved organic carbon additions. *Soil Biology and Biochemistry*, **32**, 597–601.

Majdi H, Öhrvik J (2004) Interactive effects of soil warming and fertilization on root production, mortality, and longevity in a Norway spruce stand in Northern Sweden. *Global Change Biology*, **10**, 182–188.

Manning P, Newington JE, Robson HR, et al. (2006) Decoupling the direct and indirect effects of nitrogen deposition on ecosystem function. *Ecology Letters*, **9**, 1015–1024.

Martens-Habbena W, Berube PM, Urakawa H, la Torre de JR, Stahl DA (2009) Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature*, **461**, 976–979.

Marusenko Y, Huber DP, Hall SJ (2013) Fungi mediate nitrous oxide production but not ammonia oxidation in aridland soils of the southwestern US. *Soil Biology and Biochemistry*, **63**, 24–36.

Mendum TA, Sockett RE, Hirsch PR (1999) Use of molecular and isotopic techniques to monitor the response of autotrophic ammonia-oxidizing populations of the beta subdivision of the class proteobacteria in arable soils to nitrogen fertilizer. *Applied and Environmental Microbiology*, **65**, 4155–4162.

Mothapo NV, Chen H, Cubeta MA, Shi W (2013) Nitrous oxide producing activity of diverse fungi from distinct agroecosystems. *Soil Biology and Biochemistry*, **66**, 94–101.

Mueller KE, Hobbie SE, Tilman D, Reich PB (2013) Effects of plant diversity, N fertilization, and elevated carbon dioxide on grassland soil N cycling in a long-term experiment. *Global Change Biology*, **19**, 1249–1261.

Nemani RR, Keeling CD, Hashimoto H, Jolly WM (2003) Climate-driven increases in global terrestrial net primary production from 1982 to 1999. *Science,* **300**, 1560–1563.

Niboyet A, Attard E, Barthes L, et al. (2011a) Responses of soil nitrite-oxidizers to global environmental changes. *Nature Precedings*.

Niboyet A, Barthes L, Hungate BA, et al. (2010) Responses of soil nitrogen cycling to the interactive effects of elevated CO2 and inorganic N supply. *Plant and Soil*, **327**, 35–47.

Niboyet A, Brown JR, Dijkstra P, et al. (2011b) Global Change Could Amplify Fire Effects on Soil Greenhouse Gas Emissions. *PLoS ONE*, **6**, e20105.

Niboyet A, Le Roux X, Dijkstra P, et al. (2011c) Testing interactive effects of global environmental changes on soil nitrogen cycling. *Ecosphere*, **2**, art56.

Norman JS, Barrett JE (2014) Substrate and nutrient limitation of ammonia-oxidizing bacteria and archaea in temperate forest soil. *Soil Biology and Biochemistry*, **69**, 141–146.

Offre P, Spang A, Schleper C (2012) Archaea in Biogeochemical Cycles. *Annual Review of Microbiology*, **67**, 437–457.

Okano Y, Hristova KR, Leutenegger CM, et al. (2004) Application of Real-Time PCR To Study Effects of Ammonium on Population Size of Ammonia-Oxidizing Bacteria in Soil. *Applied and Environmental Microbiology*, **70**, 1008–1016.

Olsrud M, Melillo JM, Christensen TR, Michelsen A, Wallander H, Olsson PA (2004) Response of ericoid mycorrhizal colonization and functioning to global change factors. *New Phytologist*, **162**, 459–469.

Patra AK, Abbadie L, Clays-Josserand A, et al. (2005) Effects of grazing on microbial functional groups involved in soil N dynamics. *Ecological Monographs*, **75**, 65–80.

Porras-Alfaro A, Herrera J, Natvig DO, Lipinski K, Sinsabaugh RL (2011) Diversity and distribution of soil fungal communities in a semiarid grassland. *Mycologia*, **103**, 10–21.

Ramirez KS, Craine JM, Fierer N (2012) Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, **18**, 1918–1927.

Reynolds HL, D'Antonio C (1996) The ecological significance of plasticity in root weight ratio in response to nitrogen: Opinion. *Plant and Soil*, **185**, 75–97.

Rotthauwe JH, Witzel KP, Liesack W (1997) The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Applied and Environmental Microbiology,* **63**, 4704-4712.

Röling W (2007) Do microbial numbers count? Quantifying the regulation of biogeochemical fluxes by population size and cellular activity. *FEMS Microbiology Ecology*, **62**, 202–210.

Ryden JC (1983) Denitrification loss from a grassland soil in the field receiving different rates of nitrogen as ammonium nitrate. *European Journal of Soil Science*, **34**, 355–365.

Schimel J (1995) Ecosystem Consequences of Microbial Diversity and Community Structure. In: *Arctic and alpine biodiversity: patterns, causes and ecosystem consequences* pp239–254. Berlin Heidelberg, Springer.

Schimel JP, Schaeffer SM (2012) Microbial control over carbon cycling in soil. *Frontiers in microbiology*, **3**, 348.

Shariff AR, Biondini ME, Grygiel CE (1994) Grazing intensity effects on litter decomposition and soil nitrogen mineralization. *Journal of Range Management*, **47**, 444–449.

Shaw MR (2002) Grassland Responses to Global Environmental Changes Suppressed by Elevated CO2. *Science*, **298**, 1987–1990.

Shen X-Y, Zhang L-M, Shen J-P, Li L-H, Yuan C-L, He J-Z (2011) Nitrogen loading levels affect abundance and composition of soil ammonia oxidizing prokaryotes in semiarid temperate grassland. *Journal of Soils and Sediments*, **11**, 1243–1252.

Simberloff D (2011) How common are invasion-induced ecosystem impacts? *Biological Invasions*, **13**, 1255–1268.

Smith MS, Tiedje JM (1979) Phases of denitrification following oxygen depletion in soil. *Soil Biology and Biochemistry*, **11**, 261–267.

Stahl DA, la Torre de JR (2012) Physiology and Diversity of Ammonia-Oxidizing Archaea. *Annual Review of Microbiology*, **66**, 83–101.

Steinweg JM, Dukes JS, Paul EA, Wallenstein MD (2013) Microbial responses to multi-factor climate change: effects on soil enzymes. *Frontiers in microbiology*, **4**, 146.

Stohlgren TJ, Pyšek P, Kartesz J, et al. (2011) Widespread plant species: natives versus aliens in our changing world. *Biological Invasions*, **13**, 1931–1944.

Stopnisek N, Gubry-Rangin C, Hofferle S, Nicol GW, Mandic-Mulec I, Prosser JI (2010) Thaumarchaeal ammonia oxidation in an acidic forest peat soil is not influenced by ammonium amendment. *Applied and Environmental Microbiology*, **76**, 7626–7634.

Svejcar T, Sheley R (2001) Nitrogen dynamics in perennial- and annual-dominated arid rangeland. *Journal of arid environments*, **47**, 33–46.

Swenson CF, Le Tourneau D, Erickson LC (1964) Silica in medusahead. *Weeds*, **12**, 16–18.

Šimek M, Cooper JE (2002) The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. *European Journal of Soil Science*, **53**, 345–354.

Treseder KK (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters*, **11**, 1111–1120.

Velthof GL, Oenema O (1995) Nitrous oxide fluxes from grassland in the Netherlands: II. Effects of soil type, nitrogen fertilizer application and grazing. *European Journal of Soil Science*, **46**, 541–549.

Verhamme DT, Prosser JI, Nicol GW (2011) Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. *The ISME Journal*, **5**, 1067–1071.

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry*, **13**, 87–115.

Vitousek PM, D'Antonio CM, Loope LL, Rejmanek M, Westbrooks R (1997) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, **21**, 1–16.

Vitousek PM, Walker LR, Whiteaker LD, Mueller-Dombois D, Matson PA (1987) Biological invasion by Myrica faya alters ecosystem development in Hawaii. *Science*, **238**, 802–804.

Wallace LL, Macko SA (1993) Nutrient acquisition by clipped plants as a measure of competitive success: the effects of compensation. *Functional Ecology*, **7**, 326–331.

Wallenstein MD, Myrold DD, Firestone M, Voytek M (2006) Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. *Ecological Applications*, **16**, 2143–2152.

Wardle DA, Ghani A (1995) Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? *Soil Biology and Biochemistry*, **27**, 821–828.

Weber CF, Vilgalys R, Kuske CR (2013) Changes in fungal community composition in response to elevated atmospheric CO2 and nitrogen fertilization varies with soil horizon. *Frontiers in microbiology*, **4**, 78.

Wertz S, Leigh AKK, Grayston SJ (2012) Effects of long-term fertilization of forest soils on potential nitrification and on the abundance and community structure of ammonia oxidizers and nitrite oxidizers. *FEMS Microbiology Ecology*, **79**, 142–154.

Wessén E, Hallin S (2011) Abundance of archaeal and bacterial ammonia oxidizers – Possible bioindicator for soil monitoring. *Ecological Indicators*, **11**, 1696–1698.

West AW, Sparling GP (1986) Modifications to the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents. *Journal of Microbiological Methods*, **5**, 177–189.

Zavaleta ES, Shaw MR, Chiariello NR, Mooney HA, Field CB (2003) Additive effects of simulated climate changes, elevated CO_2, and nitrogen deposition on grassland diversity. *Proceedings of the National Academy of Sciences*, **100**, 7650–7654.

Zhang L-M, Hu H-W, Shen J-P, He J-Z (2012) Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. *The ISME Journal*, **6**, 1032–1045.

Zhou X, Sherry RA, An Y, Wallace LL, Luo Y (2006) Main and interactive effects of warming, clipping, and doubled precipitation on soil CO 2efflux in a grassland ecosystem. *Global Biogeochemical Cycles*, **20**, 1–12.

Zhou Z, Shi X, Zheng Y, Qin Z, Xie D, Li Z, Guo T (2014) Abundance and community structure of ammonia-oxidizing bacteria and archaea in purple soil under long-term fertilization. *European Journal of Soil Biology*, **60**, 24–33.

3.8 Tables

Table 3-1 Effects of invasion on potential rates and N availability indices

	Nitrification Potential		Denitrification Potential		Soil NH_4^+		Soil NO_3^-		IER – NH_4^+		IER – NO_3^-	
	%	P value	%	P value	%	P value	%	P value	%	P value	%	P value
2011-12												
Treatment effects												
Invasion	- 6	0.15	**- 31**	**< 0.001**	+ 11	0.25	+ 18	0.43	+ 8	0.97	- 19	0.09
Time effects												
Time		**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**		**< 0.01**		**< 0.001**
Invasion x Time		0.45		**0.01**		0.84		0.06		0.24		0.37
2012-13												
Treatment effects												
Invasion	**- 12**	**< 0.01**	+ 4	0.17	+ 44	0.81	+ 20	0.6	+ 10	0.5	**+ 71**	**0.03**
Time effects												
Time		**< 0.001**		**0.08**		**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**
Invasion x Time		0.24		0.84		0.71		0.87		0.48		0.15

% = percent change, calculated as ((Treatment – Control)/(Control))*100. Significant responses are highlighted in bold ($P < 0.05$).

Table 3-2 Effects of nitrogen fertilization and clipping on potential rates and N availability indices

	Nitrification Potential		Denitrification Potential		Soil NH_4^+		Soil NO_3^-		IER – NH_4^+		IER – NO_3^-	
	%	P value	%	P value	%	P value	%	P value	%	P value	%	P value
2011-12												
Treatment effects												
N addition	**+ 20**	**< 0.001**	**- 1**	**0.72**	+ 1	0.98	+ 47	0.07	+ 52	0.23	**+ 443**	**< 0.001**
Clipping	**- 15**	**< 0.001**	- 4	0.99	- 7	0.40	**- 7**	**< 0.01**	**- 24**	**0.01**	- 17	0.36
N x Clipping	- 8	0.08	+ 3	0.60	- 3	0.61	- 12	0.25	- 13	0.60	+ 585	0.87
Time effects												
Time		**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**		**0.001**		**< 0.001**
N x Time		0.82		0.57		0.69		**< 0.05**		0.08		**< 0.001**
Clipping x Time		**0.05**		0.38		0.89		0.06		0.95		0.11
N x C x Time		0.59		0.20		0.81		0.94		0.55		0.65
2012-13												
Treatment effects												
N addition	**+ 25**	**< 0.001**	**- 13**	**< 0.01**	+ 11	0.57	**+ 35**	**< 0.01**	+ 7	0.23	**+ 504**	**< 0.001**
Clipping	**- 6**	**< 0.01**	**+ 20**	**< 0.05**	+ 7	0.95	**+ 2**	**0.01**	- 20	0.27	**- 11**	**< 0.001**
N x Clipping	+ 4	0.15	- 5	0.34	+ 44	0.92	+ 11	0.49	- 4	0.26	+ 336	0.85
Time effects												
Time		**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**
N x Time		0.62		**< 0.05**		0.11		0.38		0.27		**< 0.001**
Clipping x Time		0.84		0.21		0.76		0.08		0.15		0.45
N x C x Time		0.42		0.32		0.42		0.80		0.08		0.35

% = percent change, calculated as ((Treatment – Control)/(Control))*100. Significant responses are highlighted in bold ($P < 0.05$). Abbreviations: N – nitrogen, C – clipping.

Table 3-3 Soil characteristics from 0 – 15 cm depth associated with each treatment

Treatment	GWC (%)	pH	Temperature (°C)	Total C (g kg^{-1} soil)	Total N (g kg^{-1} soil)	C:N
2011-12						
Native	14.29 (1.05)	6.62 (0.03)	18.53 (1.33)	12.86 (0.28)	1.34 (0.03)	9.63 (0.05)
Invaded	14.32 (1.00)	6.62 (0.02)	22.21 (4.24)	14.4 (1.41)	1.34 (0.07)	10.62 (0.46)
N addition	14.64 (0.99)	6.58 (0.02)	17.78 (1.28)	12.54 (0.29)	1.26 (0.02)	9.97 (0.13)
Clipping	13.90 (1.00)	6.58 (0.02)	18.47 (1.35)	14.06 (1.07)	1.35 (0.05)	10.31 (0.38)
N x C	13.94 (0.89)	6.57 (0.02)	18.29 (1.30)	12.48 (0.32)	1.22 (0.03)	10.24 (0.26)
2012-13						
Native	17.07 (1.19)	6.54 (0.02)	16.53 (10.66)	12.20 (0.29)	1.24 (0.03)	9.80 (0.08)
Invaded	17.85 (1.44)	6.53 (0.02)	18.04 (10.19)	11.83 (0.26)	1.21 (0.02)	9.78 (0.10)
N addition	18.27 (1.50)	6.52 (0.02)	17.47 (9.81)	12.25 (0.27)	1.24 (0.02)	9.87 (0.13)
Clipping	18.28 (1.73)	6.53 (0.02)	20.65 (12.29)	12.25(0.49)	1.23 (0.04)	9.94 (0.10)
N x C	18.89 (1.42)	6.48 (0.02)	19.97 (12.66)	12.03 (0.38)	1.22 (0.03)	9.83 (0.13)

Values are means ± SE averaged over all four seasons (October/November – July; n = 8). GWC = gravimetric water content; pH measured in 0.01 M $CaCl_2$ solution; Temperature in 2011-12 was measured at 7.5 cm depth using a traceable metal thermometer, while temperature in 2012-13 was measured using HOBO sensors placed 7.5 cm in the soil (averages from 12:00 PM measurements on soil sampling dates 1/9/2013, 4/9/2013, 7/9/2013) ; C:N = carbon to nitrogen mass ratio of soil.

Table 3-4 Spearman's rank coefficients (r) and significance for correlations among ammonia-oxidizing bacteria and archaea and select soil parameters

AOB Abundance	November	January	April	July	All
NP	0.31	0.19	-0.03	0.30	**0.27****
Soil NH4+	-0.01	-0.10	-0.07	-0.03	0.18
Soil NO3-	0.09	0.02	0.10	0.08	-0.13
IER – NH4+	---	---	0.09	0.05	-0.03
IER – NO3-	---	---	0.08	-0.10	-0.04
pH	-0.07	0.02	-0.16	-0.14	-0.04
Temperature	---	**-0.34***	-0.21	-0.18	**-0.24****
Soil Moisture	-0.04	-0.10	-0.12	0.26	-0.09
AOA Abundance	November	January	April	July	All
NP	-0.17	-0.06	-0.18	-0.28	**-0.67*****
Soil NH4+	0.13	0.07	-0.24	**-0.66*****	0.15
Soil NO3-	-0.16	-0.01	-0.27	-0.28	**0.25****
IER – NH4+	---	---	0.02	**-0.32***	**-0.34***
IER – NO3-	---	---	-0.24	-0.25	**-0.79*****
pH	0.03	-0.02	0.18	0.32	**0.39*****
Temperature	---	-0.03	-0.30	**0.43****	**0.49*****
Soil Moisture	0.31	-0.04	**0.34***	0.26	**-0.23***
AOB / AOA	November	January	April	July	All
NP	0.25	0.14	0.12	**0.37***	**0.66*****
Soil NH4+	-0.05	0.06	0.01	**0.42****	-0.07
Soil NO3-	0.18	-0.15	0.16	0.28	**-0.21***
IER – NH4+	---	---	0.06	0.21	**0.28***
IER – NO3-	---	---	0.16	-0.07	**0.70*****
pH	-0.06	-0.06	-0.31	-0.25	**-0.31*****
Temperature	---	-0.31	-0.03	**-0.46****	**-0.65*****
Soil Moisture	-0.24	-0.09	-0.26	-0.15	**0.31*****

*** P < 0.001, ** P < 0.01, * P < 0.05, significant responses are highlighted in bold (P < 0.05). N = 40 for each individual sampling date. All = collapsed across sampling dates (N = 160; N = 80 for IER - NH_4^+/NO_3^-). NP = nitrification potential.

Table 3-5 Spearman's rank coefficients (r) and significance for correlations among nitrification and denitrification potential and select soil parameters

Nitrification Potential	2011-12					2012-13				
	October	January	April	July	All	November	January	April	July	All
Soil NH_4^+	0.20	0.19	0.18	-0.05	**0.49*** **	0.25	-0.25	-0.14	0.12	**-0.23** **
Soil NO_3^-	0.01	**0.34***	0.15	0.16	0.03	**0.55*** **	**0.42** **	**0.32***	0.00	-0.10
IER – NH_4^+	---	0.13	0.12	0.14	0.14	---	---	0.13	**0.40***	**0.37** **
IER – NO_3^-	---	0.15	0.13	0.20	**-0.26***	---	---	0.08	0.01	**0.65*** **
pH	0.18	-0.16	**0.45** **	0.25	-0.07	-0.26	-0.25	0.06	-0.20	**-0.39*** **
Temperature	---	---	---	---	---	---	-0.28	-0.26	**-0.43** **	**-0.52*** **
Soil Moisture	0.31	-0.04	**0.52*** **	-0.06	**-0.47*** **	0.02	0.03	-0.30	0.00	**0.30*** **
Microbial Biomass	0.12	**0.37***	**0.51*** **	**0.37***	**0.60*** **	**0.43** **	**0.52*** **	0.20	0.18	0.11

Denitrification Potential	2011-12					2012-13				
	October	January	April	July	All	November	January	April	July	All
Soil NH_4^+	0.29	-0.16	0.09	**0.44** **	**0.46*** **	0.13	-0.19	0.13	**0.29**	**0.20***
Soil NO_3^-	0.03	0.11	0.03	-0.25	**-0.20***	-0.13	**-0.32***	-0.01	**0.33***	-0.14
IER – NH_4^+	---	0.08	0.06	0.16	0.06	---	---	0.12	-0.03	0.07
IER – NO_3^-	---	0.04	0.08	0.11	**-0.26***	---	---	0.03	**0.36**	**-0.33***
pH	0.14	-0.11	**0.34***	0.30	-0.13	0.10	0.24	0.13	0.10	0.15
Temperature	---	---	---	---	---	---	0.23	**-0.48** **	-0.22	**0.19***
Soil Moisture	0.19	0.01	**0.40***	0.21	**-0.48*** **	**0.38***	0.11	-0.29	-0.12	**-0.19***
Microbial Biomass	**0.36***	**0.35***	0.28	**0.38***	**0.40*** **	0.20	-0.04	**0.56*** **	**0.62*** **	**0.39*** **

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, significant responses are highlighted in bold ($P < 0.05$). N = 40 for each individual sample date. All = collapsed across sampling dates within 2011-12 or 2012-13 (N = 160; N = 120 and 80 for IER - NH_4^+/NO_3^- in 2011-12 and 2012-13, respectively

3.9 Figures

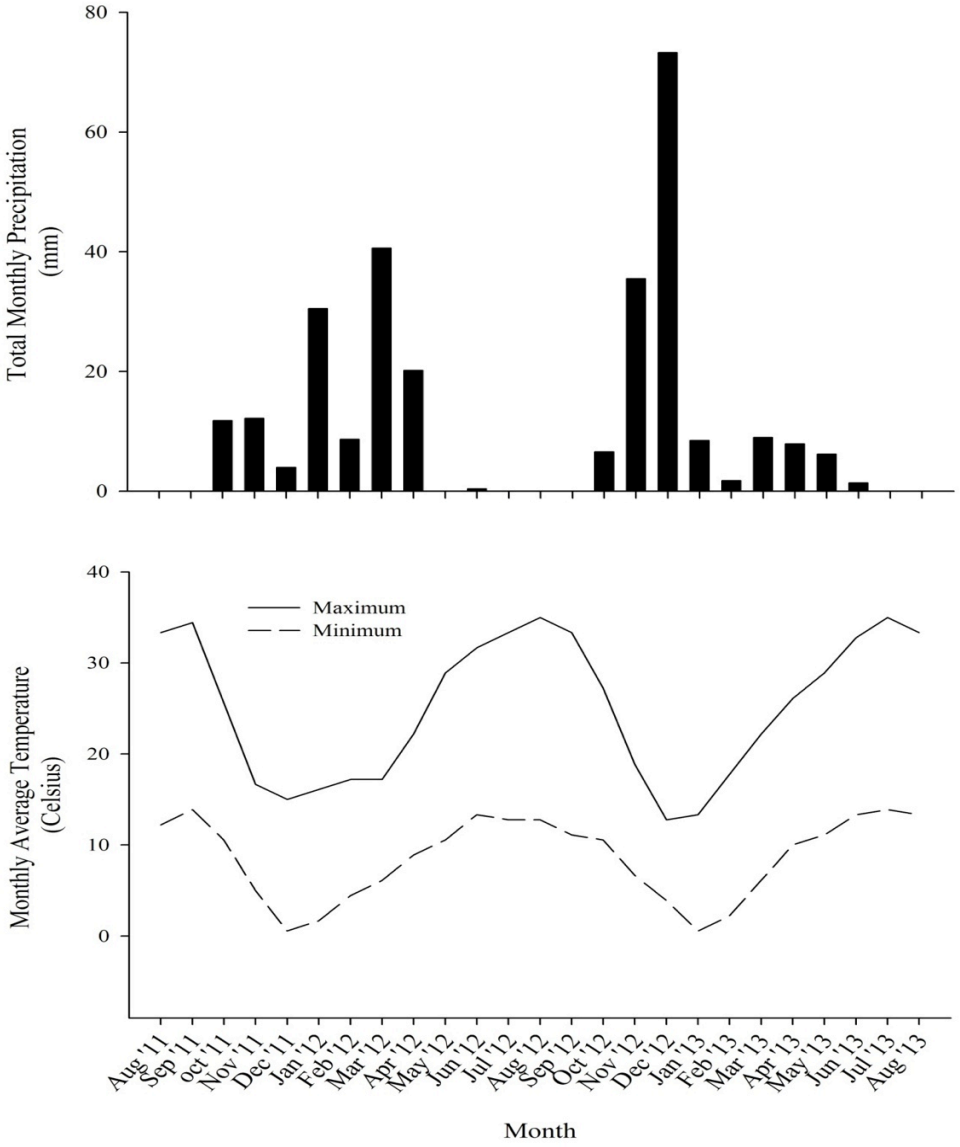

Figure 3-1 (a) Total monthly precipitation and (b) monthly maximum/minimum air temperature (2 m above ground) during the duration of our study (2011-2013). Values are derived from the University of California, Davis Climate Station (http://atm.ucdavis.edu), which is located approximately a mile away from the study site. Vertical dashed line here and in subsequent graphs represents the separation of year 1 (2011-12) and year 2 (2012-13) of sampling.

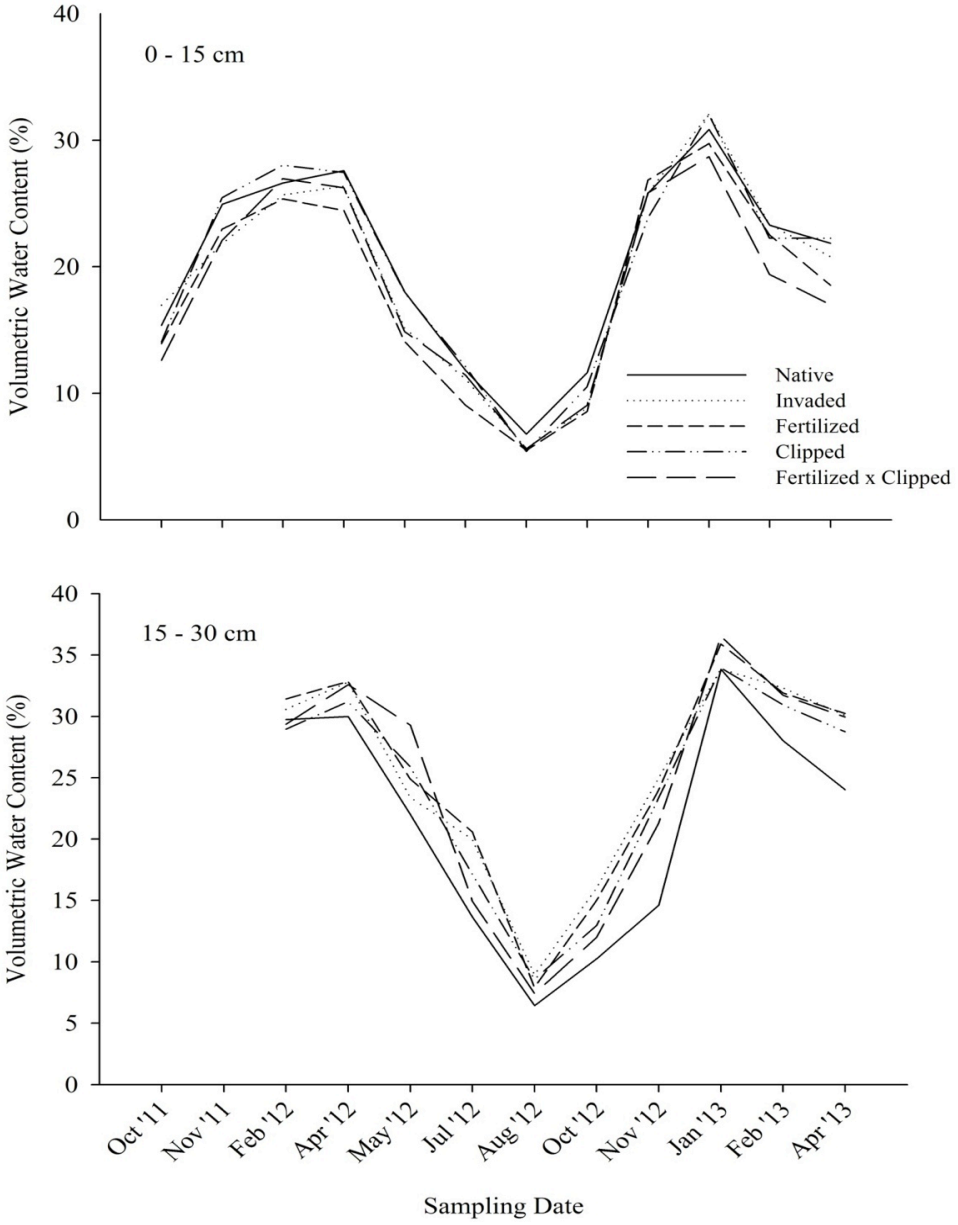

Figure 3-2 Changes in mean soil volumetric water content over time, as influenced by plant composition, fertilization, clipping, fertilization × clipping and soil depth (upper panel 0-15 cm, lower panel 15-30 cm). Values are means ± 1 SE (n = 6). Volumetric water content at both depths differed by date ($P < 0.001$), and the relationship between VWC and treatment tended to be only marginally significant ($0.05 < P < 0.1$). Volumetric water content at 15-30 cm depth is not present for October and November 2011 because 0-30 cm TDR probes were not installed at that time.

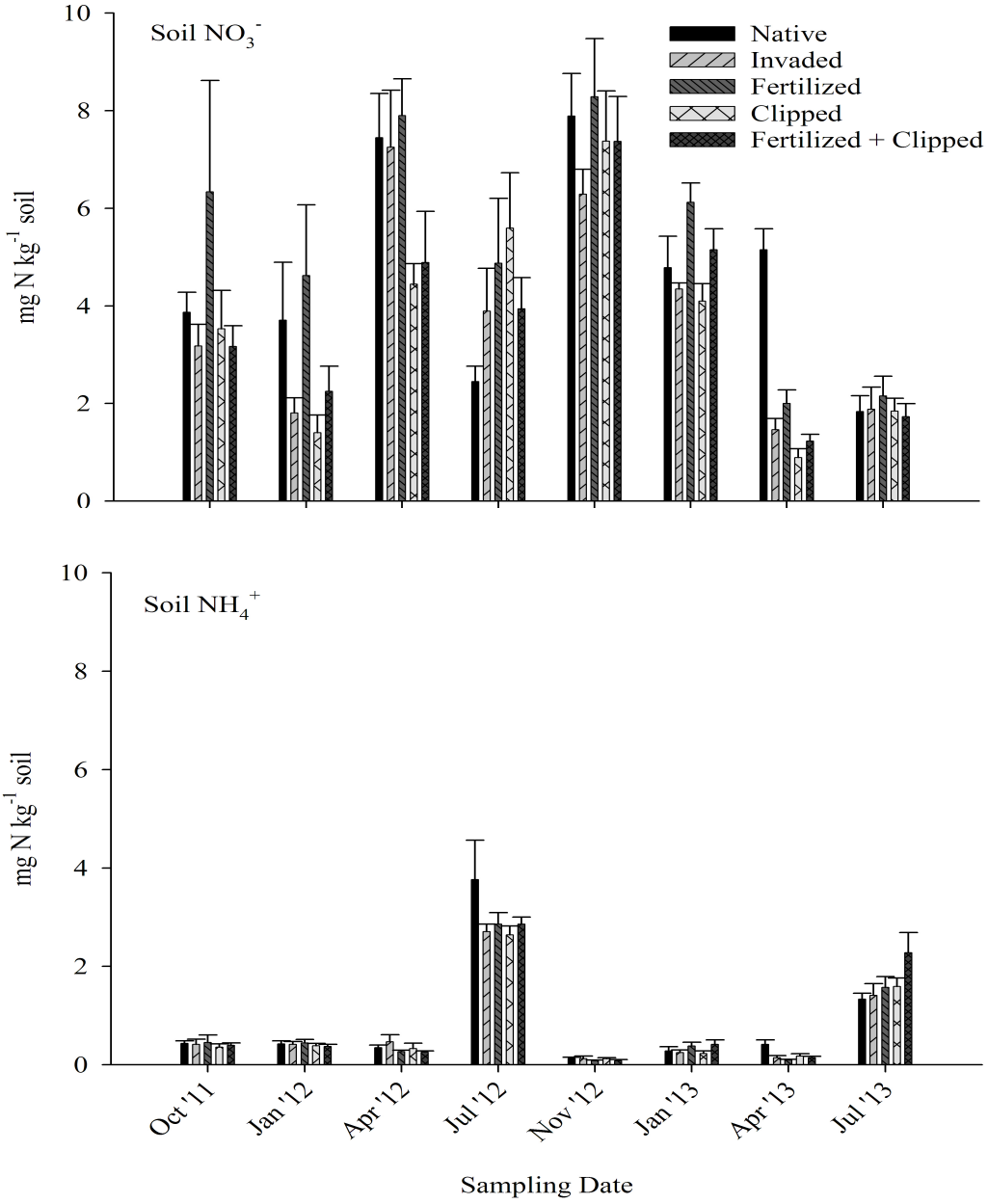

Figure 3-3 The response of soil (a) NH_4^+ and (b) NO_3^- concentrations to plant composition, fertilization, clipping, and fertilization × clipping over time. Bars ± 1 standard error of the mean (n = 8). Soil NH_4^+ differed significantly by sampling date ($P < 0.001$) but not by treatment. In 2011-12, the effect of N fertilization on soil NO_3^- was dependent upon sampling date. In 2012-13, soil NO_3^- was independently affected by treatment and date ($P < 0.01$).

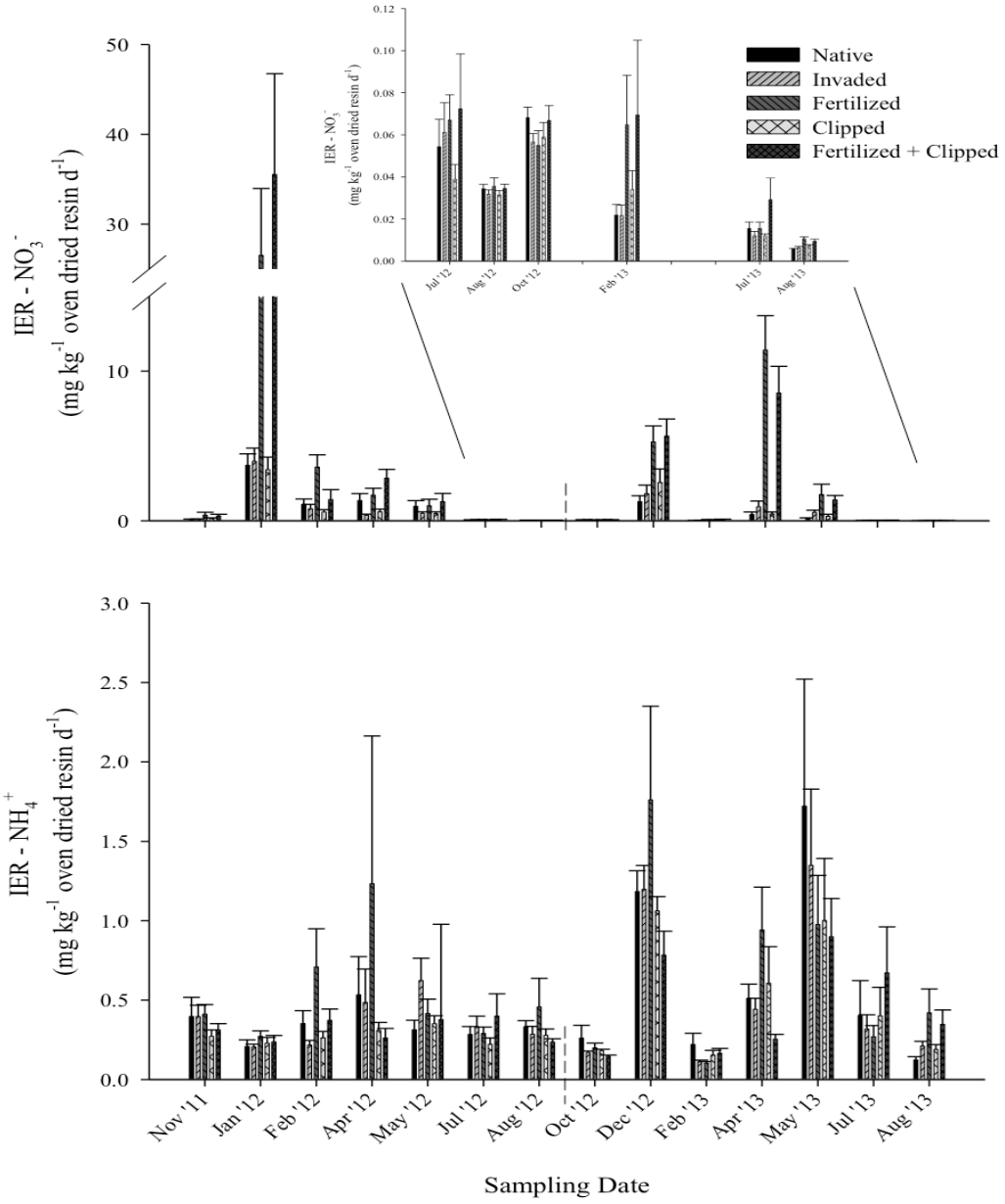

Figure 3-4 Ion-exchange resin (a) NH_4^+ and (b) NO_3^- values, as affected by plant composition, fertilization, clipping, and fertilization × clipping over time. Bars ± 1 standard error of the mean (n = 12). IER-NH4+ was significantly affected by treatment and date in 2011-12 ($P < 0.01$) and by date in 2012-13 ($P < 0.001$). IER-NO_3^- showed a significant response to treatment and date in both years. The effect of N fertilization on IER-NO_3^- was dependent upon sampling date ($P < 0.001$).

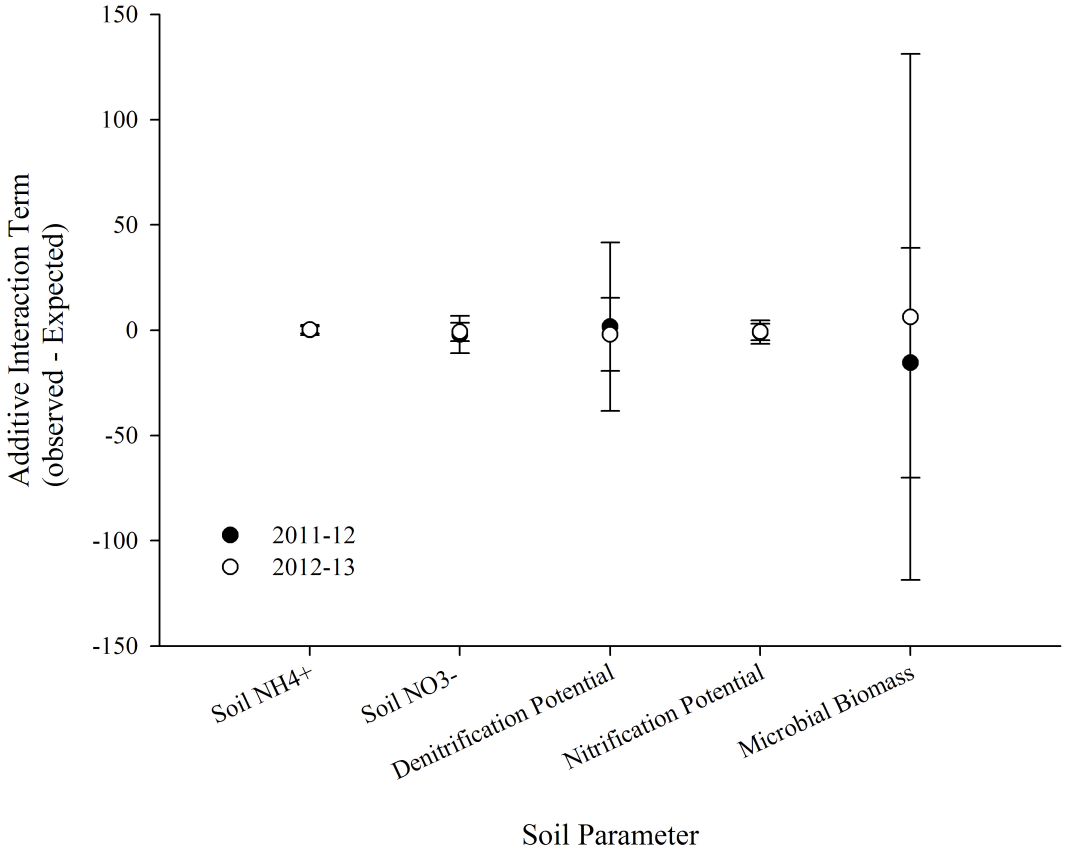

Figure 3-5 Additive interaction term, calculated as observed – expected, for five key soil measurements (mean ± 95 confidence interval). Solid circles denote measurements made in 2011-12, open circles denote those collected in 2012-13. No values deviate significantly from zero, suggesting that the interactions are additive.

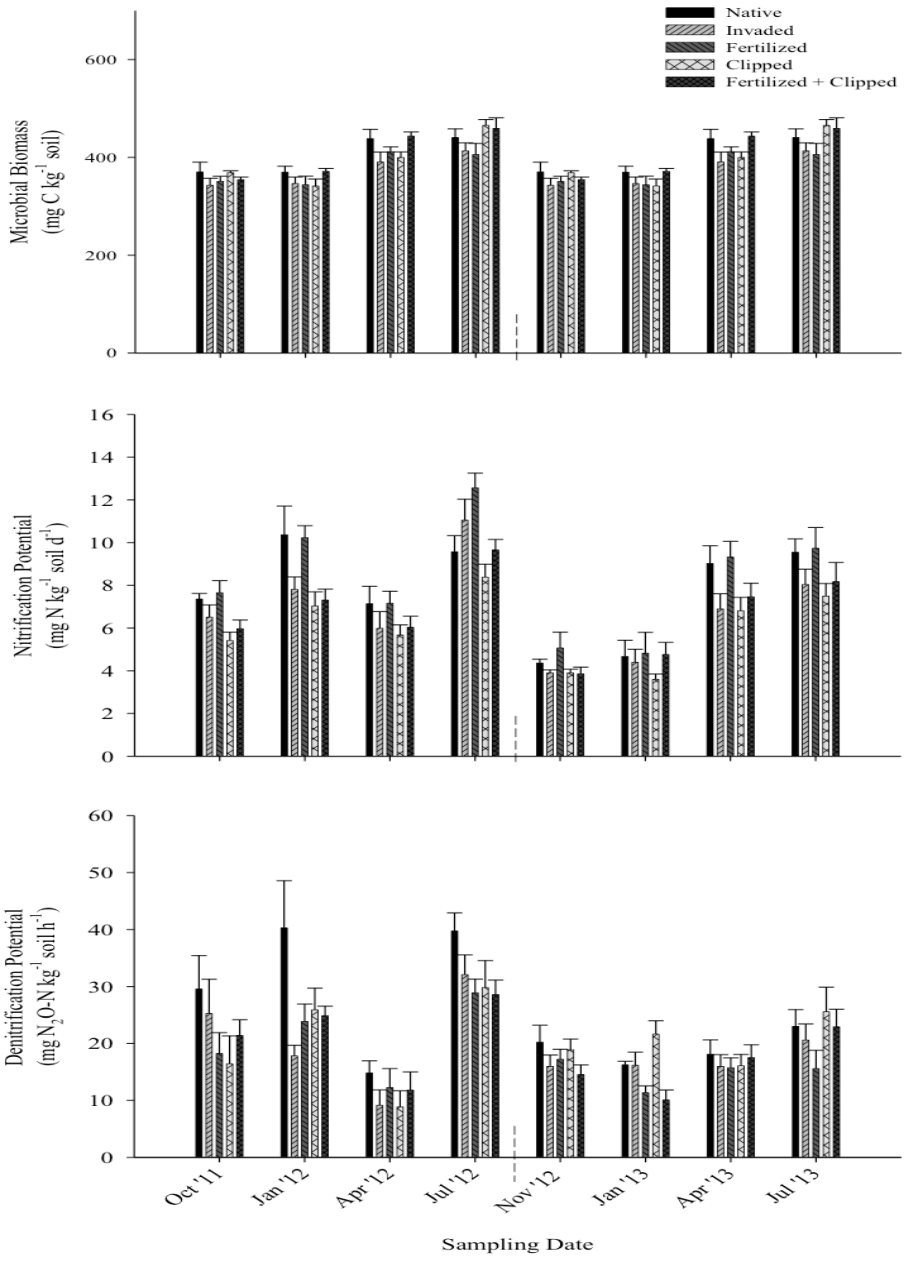

Figure 3-6 Changes in (a) microbial biomass, (b) nitrification potential, and (c) denitrification potential as influenced by plant composition, fertilization, clipping, and fertilization × clipping over time. Bars ± 1 standard error of the mean (n = 8). All three response variables differed significantly by treatment and sampling date. The effect of clipping on nitrification potential was dependent upon sampling date, as was the effect of N fertilization on denitrification potential in 2012-13 ($P < 0.05$).

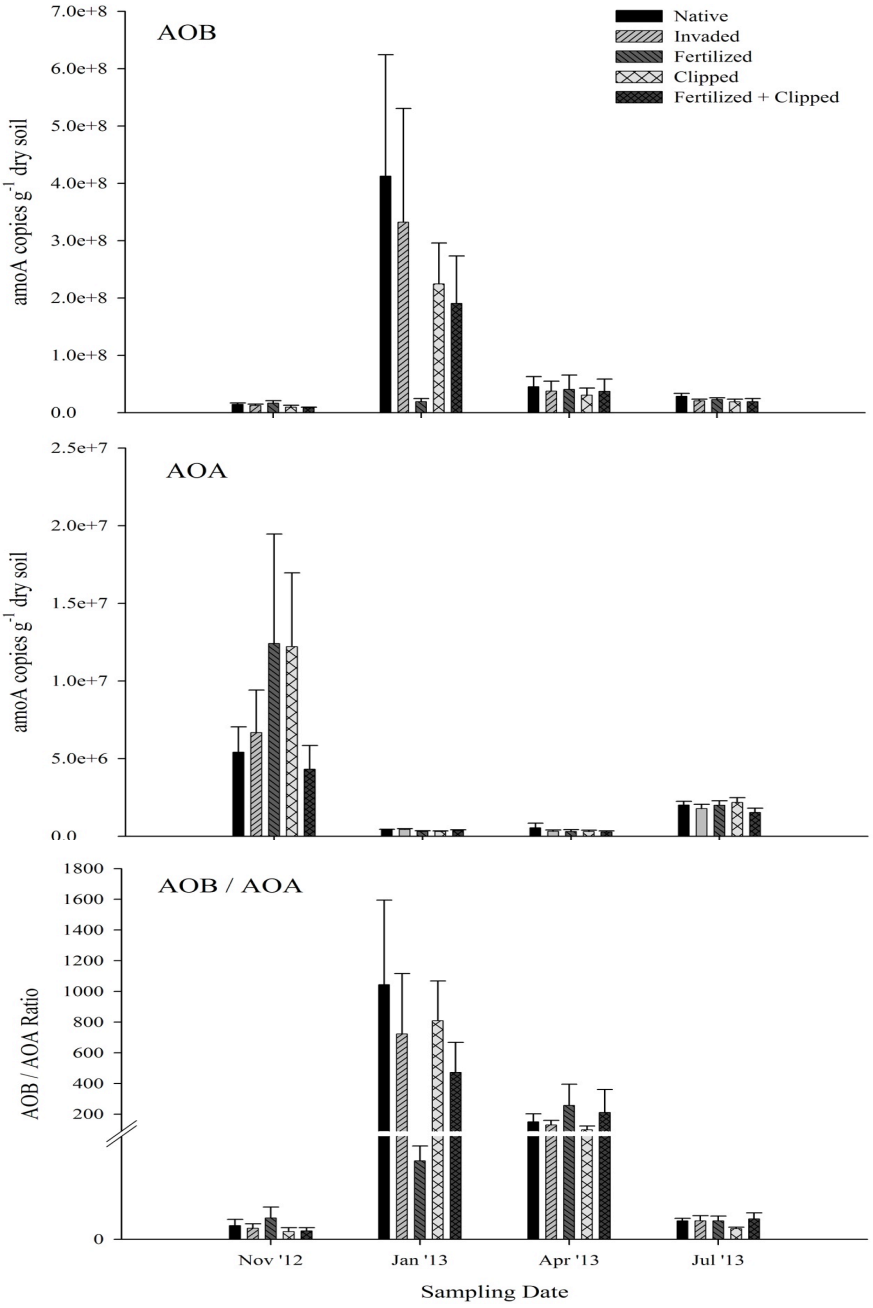

Figure 3-7 Changes in (a) AOB *amoA* gene abundance, (b) AOA *amoA* gene abundance, and (b) the ratio of AOB to AOA by treatment (native community, invaded community, fertilization, clipping, and fertilization × clipping) and sampling date. Bars ± 1 standard error of the mean (n = 8). Treatment did not affect amoA gene abundance or the ratio of AOB/AOA. All variables were significantly affected by sampling date ($P < 0.001$).

4 Soil microbial community composition is insensitive to multiple global change factors in experimental grasslands

4.1 Abstract

Soil microorganisms consume and produce trace gases, release and immobilize nutrients, and regulate vegetation dynamics. As such, microbial communities are an essential component of ecosystem response to global change. While a number of studies have sought to understand the effects of single global change factors on microbial community composition and diversity, little is known about how these factors interact. Our study used experimental grassland plots to measure the effect of three primary global changes – invasion by exotic annual grasses, N fertilization, and aboveground vegetation removal ("clipping", to simulate one component of cattle grazing) – on soil microbial community composition and diversity. We analyzed 16s rRNA using the Illumina MiSeq platform to compare bacterial and archaeal communities across treatments three (N fertilization and clipping) to five years (plant communities) after establishment. Previous work at our site showed that each individual global change factor stimulated a change in the N cycle, although the direction and magnitude across treatments varied. Given that microorganisms are intrinsically linked to N availability and rates of cycling, we expected concurrent shifts in microbial community composition and diversity across treatments. Surprisingly, bacterial and archaeal communities showed very little variation in composition and diversity, suggesting that soil microbial communities are insensitive to exotic plant invasion, N deposition, and defoliation in Mediterranean grasslands, which experience large seasonal changes in temperature, precipitation, and soil N availability. Indeed, these global environmental changes may not produce large enough effects to induce shifts in a microbial community that is already adapted to fluctuating environmental conditions.

4.2 Introduction

Positioned at the interface of the atmosphere-plant-soil system, soil microorganisms drive biogeochemical cycling from the micro- to the global-scale (Wachinger *et al.*, 2000; Falkowski *et al.*, 2008; Madsen, 2011). In their quest to obtain energy and nutrients, microbial communities decompose organic matter (Sinsabaugh *et al.*, 1991), (im)mobilize and transform nitrogen (N; Davidson *et al.*, 1990; Schimel & Bennett, 2004) and phosphorus (P; Frossard *et al.*, 2000), and consume and produce critical trace gases (e.g., CO_2, CH_4, N_2O; Conrad, 1996; Schimel & Gulledge, 1998). Additionally, soil microorganisms influence net primary production (NPP), plant community composition, and the carbon (C) storage capacity of ecosystems (Schimel & Schaeffer, 2012) by regulating the availability of nutrients (van der Heijden *et al.*, 2008) and forming (mutualistic and pathogenic) relationships with plants (Hartnett & Wilson, 1999; Klironomos *et al.*, 2000; Bever, 2003; Reynolds *et al.*, 2003; Raaijmakers *et al.*, 2009). Because of their ability to affect biogeochemical processes and vegetation dynamics, soil microbial communities likely play an important role in mediating ecosystem response to global change (Swift *et al.*, 1998; Talbot *et al.*, 2008; Zak *et al.*, 2011).

Most primary global change factors, such as N deposition and exotic plant invasion, involve changes to abiotic (e.g., soil moisture, inorganic N availability) and biotic (e.g., plant composition) variables that can directly and indirectly affect soil microbial community composition and diversity. Microorganisms vary in their functional traits (Schimel & Schaeffer, 2012; Martiny *et al.*, 2013), metabolic requirements (Falkowski et al., 2008) and response to stress (Schimel *et al.*, 2007); as a result, environmental change can favor certain groups of microorganisms over others, with possible implications for ecosystem function. For example, changes in C and N availability can affect the relative abundances of copiotrophic (r-selected; e.g., *Actinobacteria*, *Bacteriodetes* and *Proteobacteria*) and oligotrophic taxa (K-selected; e.g., *Acidobacteria* and *Verrucomicrobia*; Fierer *et al.*, 2007; Ramirez *et al.*, 2012; Fierer *et al.*, 2012). Additionally, ammonia-oxidizing bacteria (AOB) generally respond more significantly to increases in NH_4^+ availability than ammonia-oxidizing archaea (AOA; Di *et al.*, 2010), resulting in higher AOB/AOA ratios with elevated N supply (Shen *et al.*, 2011). While variations in microbial physiology at the population level provides the opportunity for differential responses to occur, it is still unclear whether this necessarily translates into broad-scale alterations of microbial community composition under all global change scenarios.

To better understand microbial responses to global change, a growing number of experiments have monitored community composition under single (Frey *et al.*, 2004; Zhang *et al.*, 2005; Batten *et al.*, 2008; Ingram *et al.*, 2008; Lesaulnier *et al.*, 2008; Campbell *et al.*, 2010; Ramirez *et al.*, 2012; Coolon *et al.*, 2013) and combined (Horz *et al.*, 2004; Chung *et al.*, 2007; Castro *et al.*, 2010; Gray *et al.*, 2011) treatments. Of these studies, many have reported significant responses in soil microbial community composition (e.g., Shade *et al.*, 2012); however, others have shown resistance to change

(Waldrop & Firestone, 2006; Cruz-Martínez *et al.*, 2009; Allison *et al.*, 2010; Marshall *et al.*, 2011; Shade *et al.*, 2012). For example, bacterial and fungal composition measured using phospholipid fatty acid analysis (PLFA) was insensitive to five years of altered plant functional identity in a grassland ecosystem (Marshall *et al.*, 2011). Furthermore, the composition of fungi subjected to warming in a boreal forest also showed no significant differences compared to the unwarmed control (Allison *et al.*, 2010). Variations in observed sensitivity may be dependent upon the global change factor, ecosystem type (Waldrop & Firestone, 2006; Shade *et al.*, 2012; Griffiths & Philippot, 2013), intensity and duration of the treatment, and physiological plasticity of the resident microorganisms (Shade *et al.*, 2012). Continued research that focuses on the response of soil microbial communities to global change factors and their interaction will help build a more comprehensive understanding of what governs microbial community sensitivity to change.

We examined soil bacterial community composition in experimental Mediterranean-type grasslands subjected to multiple global change manipulations. Treatments consisted of altered plant community composition (invaded and uninvaded grasslands), N fertilization, and defoliation, which represent three major global environmental pressures affecting grasslands worldwide (Sala *et al.*, 2000). Nitrogen fertilization and defoliation were manipulated in a factorial manner to determine whether their interaction affected soil microbial communities in an unexpected way. Given that changes in N cycling and availability had previously been demonstrated to occur in response to treatments at our site (Chapter 2), we sought to understand whether there were any accompanying changes in soil microbial community composition.

4.3 Methods

This study was conducted at an experimental site located in Davis, CA. Climatic conditions and soil classification at this site, as well as the experimental design, are described in chapter two of this dissertation.

4.3.1 Sample Collection, DNA Extraction, and Quantification

Soil samples were collected from five treatments (Native, Invaded, Fertilized, Clipped, Fertilized + Clipped ['Both']) in April 2013. Each treatment had eight replicates, where one replicate was a composite of five soil cores (2.4 cm diameter x 15 cm deep), each taken at random locations from within the plot. Composite field-moist samples were sieved (2 mm) and stored at -20 °C prior to analysis. Microbial DNA was extracted from 0.20 – 0.30 g of soil using a MO BIO PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's instructions. Extracted DNA was quantified using a Quant-iT PicoGreen ds DNA Assay Kit (Life Technologies, Carlsbad, CA, USA) and diluted to 1 ng uL^{-1} DNA.

4.3.2 Barcoded Amplicon Sequencing on the Illumina MiSeq Platform

DNA extracts (10 µL per sample) were shipped overnight on dry ice to Argonne National Laboratory (Lemont, Illinois, USA) for PCR amplification and sequencing using the Illumina MiSeq2000 platform. The PCR amplification of 16S rRNA was conducted on triplicate samples using primers (F515/R806 barcoded) targeting the V4 region (Caporaso *et al.*, 2012). Cycle conditions followed the Earth Microbiome Project protocol (Caporaso *et al.*, 2011): 3 min @ 94 °C (denaturation); 35 cycles of 45 s @ 94 °C, 60 s @ 50 °C, 90 s @ 72 °C (amplification); and 10 min @ 72 °C (extension). Each 25 µL reaction mixture included 10 µL 5 PRIME Hot Master Mix (5 PRIME, Inc., Gaithersburg, MD, USA), 0.2 µM forward and reverse primers, 1 µL template DNA, and 13 µL PCR grade water (MoBio Laboratories, Inc., Carlsbad, CA, USA). After amplification, the triplicate PCR products were combined for each sample into a single volume, which was subsequently run on an agarose gel and quantified using a Quant-iT PicoGreen ds DNA Assay Kit. Equal amounts of amplicon from each sample were pooled, purified using a MoBio UltraClean PCR Clean-Up Kit, and sequenced using the Illumina MiSeq2000 platform.

4.3.3 Sequence Analysis

We used default parameters in Quantitative Insights into Microbial Ecology (QIIME; Caporaso *et al.,* 2010) for quality control. Specifically, reads were excluded if there were more than three consecutive low quality base calls, if less than 75% of the read length was consecutive high quality base calls, if a Phred score was below three, if one or more ambiguous calls were present, or if the length was less than 75 bases (Bokulich *et al.,* 2013). After forward and reverse reads were joined and demultiplexed, we picked operational taxonomic units (OTU) at 97% similarity using open reference UCLUST against the 13_8 release of the Greengenes database. Reads that did not match any sequences in the database were clustered *de novo*.

Prior to conducting diversity analyses, OTUs were rarefied to 12,000 reads per sample. Alpha diversity was measured using a variety of approaches: Shannon diversity index, Chao1 richness, Simpson's D, and Faith's phylogenetic diversity. Diversity indices were correlated with select soil characteristics (NH_4^+, NO_3^-, microbial biomass [SIR], pH, and soil moisture) using Spearman's rank correlation. Beta diversity was visualized using principle coordinate analysis (PCoA) from unweighted and weighted Unifrac distances; analysis of similarities (ANOSIM) and perMANOVA were used to determine if beta diversity differed significantly among treatments. Unifrac uses the overlap in branch lengths to estimate phylogenetic distance, or relatedness, between pairs of bacterial communities (Lozupone & Knight, 2005). Treatment effects on the relative abundances of select taxa were examined using analysis of variance (ANOVA, $\alpha = 0.05$). Interactions between N fertilization and clipping were determined by comparing expected additive effects to observed effects (same methods as Chapter 2). Models were checked for normality using a Shapiro-Wilk test and were log transformed when necessary. In addition, we utilized a distance-based redundancy analysis (db-RDA; Legendre &

Anderson, 1999) to determine which soil characteristics best explained the variation in microbial community composition across treatments.

4.4 Results

Sequencing of 40 samples (5 treatments with 8 replicates each) resulted in approximately 2.1 million reads. After quality control, 1.1 million reads remained, averaging 29,441 reads per sample (average sequence length = 253 bp). All samples were subsampled to 12,000 reads prior to running diversity analyses (Figure 4-1).

The dominant phyla were similar across all treatments and replicates (Figure 4-2), with *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacteriodetes,* and *Gemmatimonadetes* accounting for approximately 80% of sequences from each sample (range 80 – 82%). There were 26 additional phyla that were present in all soils but at lower abundances, and 10 other low-abundance phyla that were not consistently present across soils (Figure 4-3). The interaction of N fertilization and clipping affected the relative abundances of individual bacterial and archaeal taxa more often than main treatment effects (Table 4-1; Figure 4-4). Of the top 10 most abundant phyla (Fig. 2), two showed marginally significant responses to main treatment effects: *Proteobacteria* ($P = 0.07$) were reduced and *Verrucomicrobia* ($P = 0.09$) were increased in clipped relative to unclipped treatments. In contrast, neither N fertilization nor clipping individually affected *Actinobacteria, Planctomycetes, Chloroflexi,* and *Crenarchaeota*; however, these phyla responded antagonistically to the interactions of N fertilization and clipping (N fertilization x Clipping, $P < 0.05$).

The relative abundances of nitrogen-cycling microorganisms were also compared. *Nitrosomonadaceae*, a family containing ammonia-oxidizing bacteria, was significantly reduced in invaded relative to native treatments ($P < 0.05$, Figure 4-5) and demonstrated a marginally significant reduction with clipping ($P = 0.09$). In addition, *Nitrososphaeraceae*, a family of ammonia-oxidizing archaea, was affected by the interaction of N fertilization and clipping (Table 4-1), while *Nitrospirae*, a phylum of nitrite oxidizing bacteria, was also detected but showed no significant response to treatment.

Bacterial and archaeal community diversity and overall composition were unaffected by the global change factors manipulated in our study. Specifically, community richness, evenness, and phylogenetic diversity were statistically indistinguishable among treatments, and did not correlate with any of the soil characteristics listed in Table 4-2. Principle Coordinates Analysis of the UniFrac distance illustrated a lack of distinct clustering by treatment (Figure 4-6), which was confirmed using ANOSIM ($R = 0.04$, $P = 0.09$, permutations = 999), perMANOVA (Pseudo-F statistic = 1.01, $P = 0.23$, permutations = 999), and ADONIS ($R2 = 0.11$, $P = 0.23$, permutations = 999). Additionally, select soil characteristics (available N, soil pH, and soil moisture) failed to explain any variation in microbial community composition ($P = 0.46$, permutations = 99).

4.5 Discussion

Using a well-replicated field experiment, we asked whether microbial community composition was affected by changes in plant community composition, aboveground vegetation removal ('clipping'), and N fertilization in a Mediterranean-type grassland. The vegetation treatments in our study represented grassland communities native to California, and grassland communities (native and naturalized species) invaded by two exotic species, *Aegilops triuncialis* and *Elymus caput-medusae*. Invaded communities were subjected to clipping and N fertilization in order to simulate aboveground vegetation removal via livestock grazing and N deposition, which are two global change factors that affect California grasslands simultaneously. Our results demonstrate that microbial composition was largely insensitive to each treatment, suggesting a great deal of resistance or resilience in these soil microbial communities.

We found no differences in broad-scale microbial community composition or diversity in invaded compared to native plant communities. This was unexpected, given that changes in some abiotic soil characteristics (e.g., inorganic N, water availability, temperature) were observed, which could affect soil microbial community composition (Wallenstein *et al.,* 2006; Ramirez *et al.,* 2012). We did find that the relative abundance of *Nitrosomondaceae,* a family of ammonia-oxidizing bacteria, was reduced with invasion, which is consistent with reductions in nitrification potential in these treatments. However, the relative abundance of *Nitrosomonadaceae* failed to significantly explain variation in potential rates of nitrification (Linear regression; $R^2 = 0.01$, $P = 0.28$; $N = 8$); this could be due to differences in per cell nitrification rates and varying contributions of other ammonia oxidizing microorganisms, such as ammonia oxidizing archaea.

There are at least three reasons that could explain the lack of response in microbial community composition to invasion. First, it is possible that the effects of invasion were diluted, given that we compared native communities to mixed communities comprised of native, naturalized, and invasive plants. Second, the treatments may not have been established long enough to induce changes in microbial community composition. Potthoff *et al.*, (2006) found that soil microbial communities were similar between exotic annual and restored native perennial grasslands, and suggested this had to do with the relatively recent establishment (four years) of the perennial bunchgrasses. Finally, microbial communities at our site may be resistant or resilient to global change.

Our results agree with a number of studies that have found small or insignificant effects of plant identity on microbial community composition compared to other variables such as soil type (Buyer & Roberts, 2002; Singh *et al.*, 2007) or plant productivity (Marshall *et al.*, 2011; De Deyn *et al.*, 2011), but contrast with studies that have demonstrated significant responses in microbial composition to exotic plant invasion (Belnap & Phillips, 2001; Hawkes *et al.*, 2005; Batten *et al.*, 2006; 2008; Zhang *et al.*, 2013). Together, the inconsistency in effects between our study and others suggest that the impact of plant invasions on soil microbial communities may be dependent upon site,

functional differences between the native and invasive species (Vilà et al., 2011), and duration of the invasion process (Kulmatiski & Beard, 2011).

In contrast to other studies that tend to focus on the immediate or short-term response of microbial communities to defoliation (Mawdsley & Bardgett, 1997; Bardgett *et al.*, 1998), we observed minor effects of three years of clipping on microbial community composition. Indeed, microbial diversity remained unaffected, although clipping reduced the abundance of *Proteobacteri*a and increased *Verrucomicrobia,* which are copiotrophic and oligotrophic taxa, respectively (Fierer et al., 2007; Nemergut et al., 2008). There are a number of mechanisms by which defoliation can influence soil microbial communities, including changes in soil temperature, moisture, and nutrient and C availability (Bardgett *et al.*, 1998; De Graaff *et al.*, 2010). Previous work at our site demonstrated reduced N availability in clipped treatments (Chapter 2), which likely contributed to the relative increase in *Proteobacteri*a and decrease in *Verrucomicrobia.* Despite these rather minor changes, our results suggest that microbial community composition of invaded grasslands is largely insensitive to aboveground vegetation removal.

Our results that N fertilization did not significantly change in microbial community composition or diversity were surprising. Elevated N supply is a particularly well-studied global change factor, and has been shown to reduce microbial diversity and alter composition by favoring copiotrophic phyla such as *Actinobacteria* and *Firmicutes* (Nemergut *et al.*, 2008; Campbell *et al.*, 2010; Ramirez *et al.*, 2012; Fierer *et al.*, 2012; Coolon *et al.*, 2013). We had previously shown drastic changes in N availability (up to 504% increase) and potential rates of cycling with fertilization (Chapter 2); as such, we expected to observe increases in the relative abundances of copiotrophic taxa, leading to overall shifts in community composition and reduced diversity. Instead, our results indicate that changes in N availability and rates of cycling occur independently of changes in broad-scale community composition, and so likely represent microbial physiological response to change.

The observed insensitivity of microbial communities to each treatment may be due to the large seasonal variability at our site. California grasslands experience a Mediterranean climate that is marked by strong seasonal changes in soil N availability, moisture, and temperature – changes that are large compared to those observed between treatments (Figure 4-7). Microorganisms within this environment should be adapted to cope with significant seasonal changes in edaphic variables – for example, through individual metabolic capabilities, gene expression, and mixotrophy (Shade *et al.* 2012). As a result, the community is able to withstand comparatively minor effects of treatment without changing composition. Indeed, Waldrop & Firestone, (2006) found that microbial communities in grassland soils were insensitive to change when transplanted for two years into an oak-dominated environment, and concluded that this was likely because the new environmental conditions were not outside the "life history" of the microbial community. Cruz-Martínez *et al.*, (2009) also found that seasonal changes in microbial composition trumped the effects of altered precipitation at any single sampling date in a

coastal grassland, lending further evidence to the idea that microorganisms in Mediterranean soils have the physiological capability to withstand compositional changes, at least in response to experimental global change treatments. Differences in background variation experienced by resident microorganisms may explain the discrepancy between our results and other studies that reported significant changes in response to global change factors.

Experimental limitations may have also contributed to the lack of response observed in microbial communities. It is possible that the effects of fertilization and clipping (which can be considered as "pulse" events) were transient and disappeared before our sampling effort. If this were the case, the microbial community would actually be resilient, not resistant to change. In addition, the effect of treatment may be dependent upon time, so sampling at more than one time point would increase the likelihood of picking up compositional differences within microbial communities. However, the influence of treatment on microbial activity (potential nitrification and denitrification) and edaphic variables was rarely dependent upon time, so this possibility seems unlikely (Chapter 2). Finally, it is possible that we would have captured ecologically relevant changes in microbial composition by sequencing key functional genes, such as *amoA* (ammonia oxidation) or nirS and nirK (denitrification), instead of using a universal gene such as 16 rRNA.

Overall, microbial communities in Mediterranean grassland soils appear to be insensitive to multiple global change factors. While individual taxa showed some variation by treatment, overall community composition and diversity did not change. The lack of sensitivity observed in our study may be a result of the historical exposure of microorganisms in these soils to large seasonal variations in key edaphic factors. These results, coupled with prior work at this site demonstrating large changes in N cycling by treatment (Chapter 2), suggest that microbial functioning may be decoupled from composition in these soils.

4.6 References

Allison SD, McGuire KL, Treseder KK (2010) Resistance of microbial and soil properties to warming treatment seven years after boreal fire. *Soil Biology and Biochemistry*, **42**, 1872–1878.

Bardgett RD, Wardle DA, Yeates GW (1998) Linking above-ground and below-ground interactions: How plant responses to foliar herbivory influence soil organisms. *Soil Biology and Biochemistry*, **30**, 1867–1878.

Batten KM, Scow KM, Espeland EK (2008) Soil microbial community associated with an invasive grass differentially impacts native plant performance. *Microbial Ecology*, **58**, 220–228.

Batten KM, Scow KM, Davies KF, Harrison SP (2006) Two Invasive Plants Alter Soil

Microbial Community Composition in Serpentine Grasslands. *Biological Invasions*, **8**, 217–230.

Belnap J, Phillips SL (2001) Soil biota in an ungrazed grassland: response to annual grass (Bromus tectorum) invasion. *Ecological Applications*, **11**, 1261–1275.

Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist*, **157**, 465–473.

Bokulich NA, Subramanian S, Faith JJ, et al. (2013) Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods*, **10**, 57–59.

Buyer JS, Roberts DP, Russek-Cohen E (2002) Soil and plant effects on microbial community structure - Canadian Journal of Microbiology. *Canadian Journal of Microbiology*, **48**, 955–964.

Campbell BJ, Polson SW, Hanson TE, Mack MC, Schuur EAG (2010) The effect of nutrient deposition on bacterial communities in Arctic tundra soil. *Environmental Microbiology*, **12**, 1842–1854.

Caporaso JG, Kuczynski J, Stombaugh J, et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, **7**, 335–336.

Caporaso JG, Lauber CL, Walters WA, et al. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, **6**, 1621–1624.

Caporaso JG, Lauber CL, Walters WA, et al. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, **108**, 4516–4522.

Castro HF, Classen AT, Austin EE, Norby RJ, Schadt CW (2010) Soil Microbial Community Responses to Multiple Experimental Climate Change Drivers. *Applied and Environmental Microbiology*, **76**, 999–1007.

Chung H, Zak DR, Reich PB (2007) Plant species richness, elevated CO2, and atmospheric nitrogen deposition alter soil microbial community composition and function. *Global Change Biology,* **13**, 980–989.

Conrad R (1996) Soil microorganisms as controllers of atmospheric trace gases (H2, CO, CH4, OCS, N2O, and NO). *Microbiological reviews*, **60**, 609–640.

Coolon JD, Jones KL, Todd TC, Blair JM, Herman MA (2013) Long-Term Nitrogen Amendment Alters the Diversity and Assemblage of Soil Bacterial Communities in Tallgrass Prairie. *PLoS ONE*, **8**, e67884.

Cruz-Martínez K, Suttle KB, Brodie EL, Power ME, Andersen GL, Banfield JF (2009) Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. *The ISME Journal*, **3**, 738–744.

Davidson EA, Stark JM, Firestone MK (1990) Microbial production and consumption of nitrate in an annual grassland. *Ecology*, **71**, 1968–1975.

De Deyn GB, Quirk H, Bardgett RD (2011) Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. *Biology Letters*, **7**, 75–78.

De Graaff MA, Classen AT, Castro HF, Schadt CW (2010) Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. *New Phytologist*, **188**, 1055–1064.

Di HJ, Cameron KC, Shen J-P, Winefield CS, O'Callaghan M, Bowatte S, He J-Z (2010) Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. *FEMS Microbiology Ecology*, **72**, 386–394.

Falkowski PG, Fenchel T, Delong EF (2008) The microbial engines that drive Earth's biogeochemical cycles. *Science*, **320**, 1034–1039.

Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. *Ecology*, **88**, 1354–1364.

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *The ISME Journal*, **6**, 1007–1017.

Frey SD, Knorr M, Parrent JL, Simpson RT (2004) Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management*, **196**, 159–171.

Frossard E, Condron LM, Oberson A, Sinaj S, Fardeau JC (2000) Processes Governing Phosphorus Availability in Temperate Soils. *Journal of Environment Quality*, **29**, 15–23.

Gray SB, Classen AT, Kardol P, Yermakov Z, Miller M (2011) Multiple Climate Change Factors Interact to Alter Soil Microbial Community Structure in an Old-Field Ecosystem. *Soil Science Society of America Journal*, **75**, 2217–2226.

Griffiths BS, Philippot L (2013) Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiology Reviews*, **37**, 112–129.

Hartnett DC, Wilson GWT (1999) Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology*, **80**, 1187–1195.

Hawkes CV, Wren IF, Herman DJ, Firestone MK (2005) Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecology Letters*, **8**, 976–985.

Horz H-P, Barbrook A, Field CB, Bohannan BJM (2004) Ammonia-oxidizing bacteria respond to multifactorial global change. *Proceedings of the National Academy of Sciences*, **101**, 15136–15141.

Ingram LJ, Stahl PD, Schuman GE, et al. (2008) Grazing Impacts on Soil Carbon and Microbial Communities in a Mixed-Grass Ecosystem. *Soil Science Society of America Journal*, **72**, 939–948.

Klironomos JN, McCune J, Hart M, Neville J (2000) The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecology Letters*, **3**, 137–141.

Kulmatiski A, Beard KH (2011) Long-term plant growth legacies overwhelm short-term plant growth effects on soil microbial community structure. *Soil Biology and Biochemistry*, **43**, 823–830.

Legendre P, Anderson MJ (1999) Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs*, **69**, 1–24.

Lesaulnier C, Papamichail D, McCorkle S, et al. (2008) Elevated atmospheric CO 2affects soil microbial diversity associated with trembling aspen. *Environmental Microbiology*, **10**, 926–941.

Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, **71**, 8228–8235.

Madsen EL (2011) Microorganisms and their roles in fundamental biogeochemical cycles. *Current opinion in biotechnology*, **22**, 456–464.

Marshall CB, McLaren JR, Turkington R (2011) Soil microbial communities resistant to changes in plant functional group composition. *Soil Biology and Biochemistry*, **43**, 78–85.

Martiny AC, Treseder K, Pusch G (2013) Phylogenetic conservatism of functional traits in microorganisms. *The ISME Journal*, **7**, 830–838.

Mawdsley JL, Bardgett RD (1997) Continuous defoliation of perennial ryegrass (Lolium perenne) and white clover (Trifolium repens) and associated changes in the composition and activity of the microbial population of an upland grassland soil. *Biology and Fertility of Soils*, **24**, 52–58.

Nemergut DR, Townsend AR, Sattin SR, et al. (2008) The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: implications for carbon and nitrogen cycling. *Environmental Microbiology,* **10**, 3093–3105.

Potthoff M, Steenwerth KL, Jackson LE, Drenovsky RE, Scow KM, Joergensen RG (2006) Soil microbial community composition as affected by restoration practices in California grassland. *Soil Biology and Biochemistry*, **38**, 1851–1860.

Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*, **321**, 341–361.

Ramirez KS, Craine JM, Fierer N (2012) Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, **18**, 1918–1927.

Reynolds HL, Packer A, Bever JD, Clay K (2003) Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology*, **84**, 2281–2291.

Sala OE, Chapin FS, Armesto JJ, et al. (2000) Global biodiversity scenarios for the year 2100. *Science*, **287**, 1770–1774.

Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. *Ecology*, **88**, 1386–1394.

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. *Ecology*, **85**, 591–602.

Schimel JP, Gulledge J (1998) Microbial community structure and global trace gases. *Global Change Biology*, **4**, 745–758.

Schimel JP, Schaeffer SM (2012) Microbial control over carbon cycling in soil. *Frontiers in microbiology*, **3**, 348.

Shade A, Peter H, Allison SD, et al. (2012) Fundamentals of Microbial Community Resistance and Resilience. *Frontiers in microbiology*, **3**, 1–18.

Shen X-Y, Zhang L-M, Shen J-P, Li L-H, Yuan C-L, He J-Z (2011) Nitrogen loading levels affect abundance and composition of soil ammonia oxidizing prokaryotes in semiarid temperate grassland. *Journal of Soils and Sediments*, **11**, 1243–1252.

Singh BK, Munro S, Potts JM, Millard P (2007) Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. *Applied Soil Ecology*, **36**, 147–155.

Sinsabaugh RL, Antibus RK, Linkins AE (1991) An enzymic approach to the analysis of microbial activity during plant litter decomposition. *Agriculture, Ecosystems & Environment*, **34**, 43–54.

Swift MJ, Andren O, Brussaard L, et al. (1998) Global change, soil biodiversity, and nitrogen cycling in terrestrial ecosystems: three case studies. *Global Change Biology*, **4**, 729–743.

Talbot JM, Allison SD, Treseder KK (2008) Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Functional Ecology*, **22**, 955–963.

van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, **11**, 296–310.

Vilà M, Espinar JL, Hejda M, et al. (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters*, **14**, 702–708.

Wachinger G, Fiedler S, Zepp K, Gattinger A (2000) Variability of soil methane production on the micro-scale: spatial association with hot spots of organic material and Archaeal populations. *Soil Biology and Biochemistry,* **32**, 1121-1130.

Waldrop MP, Firestone MK (2006) Response of Microbial Community Composition and Function to Soil Climate Change. *Microbial Ecology*, **52**, 716–724.

Wallenstein MD, Myrold DD, Firestone M, Voytek M (2006) Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. *Ecological Applications*, **16**, 2143–2152.

Zak DR, Pregitzer K, Burton AJ, Edwards IP, Kellner H (2011) Microbial responses to a changing environment: implications for the future functioning of terrestrial ecosystems. *Fungal Ecology*, **4**, 386–395.

Zhang W, Parker KM, Luo Y, Wan S, Wallace LL, Hu S (2005) Soil microbial responses to experimental warming and clipping in a tallgrass prairie. *Global Change Biology*, **11**, 266–277.

Zhang Y, Wang L, Xie X, Huang L, Wu Y (2013) Ecological Engineering. *Ecological Engineering*, **58**, 77–83.

4.7 Tables

Table 4-1 Main and interactive effects of N fertilization and clipping on the top 10 dominant phyla across samples and nitrifying taxa. Significant ($P < 0.05$) and marginally significant ($P < 0.1$) values are highlighted in bold.

	N addition	Clipping	N addition x Clipping
		P-value	
(a) Dominant Phyla			
Proteobacteria	0.39	**0.07**	0.33
Actinobacteria	0.93	0.53	**0.01**
Bacteriodetes	0.59	0.75	0.14
Acidobacteria	0.17	0.36	0.32
Gemmatimonadetes	0.41	0.64	0.83
Verrucomicrobia	0.73	**0.09**	0.35
Firmicutes	0.13	0.92	0.17
Planctomyetes	0.36	0.99	**0.05**
Chloroflexi	0.97	0.98	**0.00**
Crenarchaeota	0.52	0.42	**0.00**
(b) Nitrifiers			
Nitrospirae	0.17	0.98	0.53
Nitrososphaeraceae	0.69	0.29	**0.00**
Nitrosomonadaceae	0.84	**0.09**	0.97

Table 4-2 Spearman's rank correlations (r) between diversity indices and soil characteristics (n = 39). No significant correlations were observed. SIR = substrate induced respiration (measure of microbial biomass); SM = soil moisture.

	NH_4^+	NO_3^-	SIR	pH	SM
Chao 1	0.00	0.19	-0.07	-0.03	0.07
Shannon	-0.02	0.08	-0.08	0.06	0.11
Simpson's	-0.21	-0.14	-0.12	0.23	0.14
Faith's Phylogeny	-0.07	-0.21	-0.07	0.04	0.15

4.8 Figures

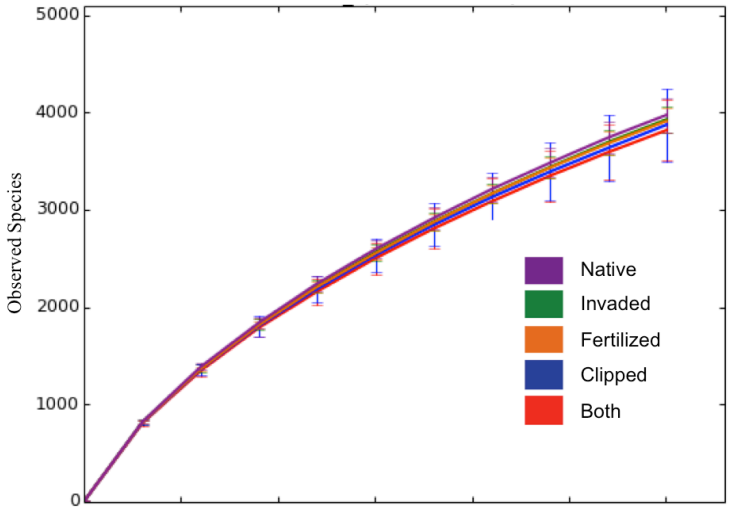

Figure 4-1 Rarefaction curve of OTU richness by treatment (purple – native community; green – invaded community; orange – fertilized; blue – clipped; red – fertilized x clipped). Samples were rarefied to the lowest common denominator: 12,000 reads.

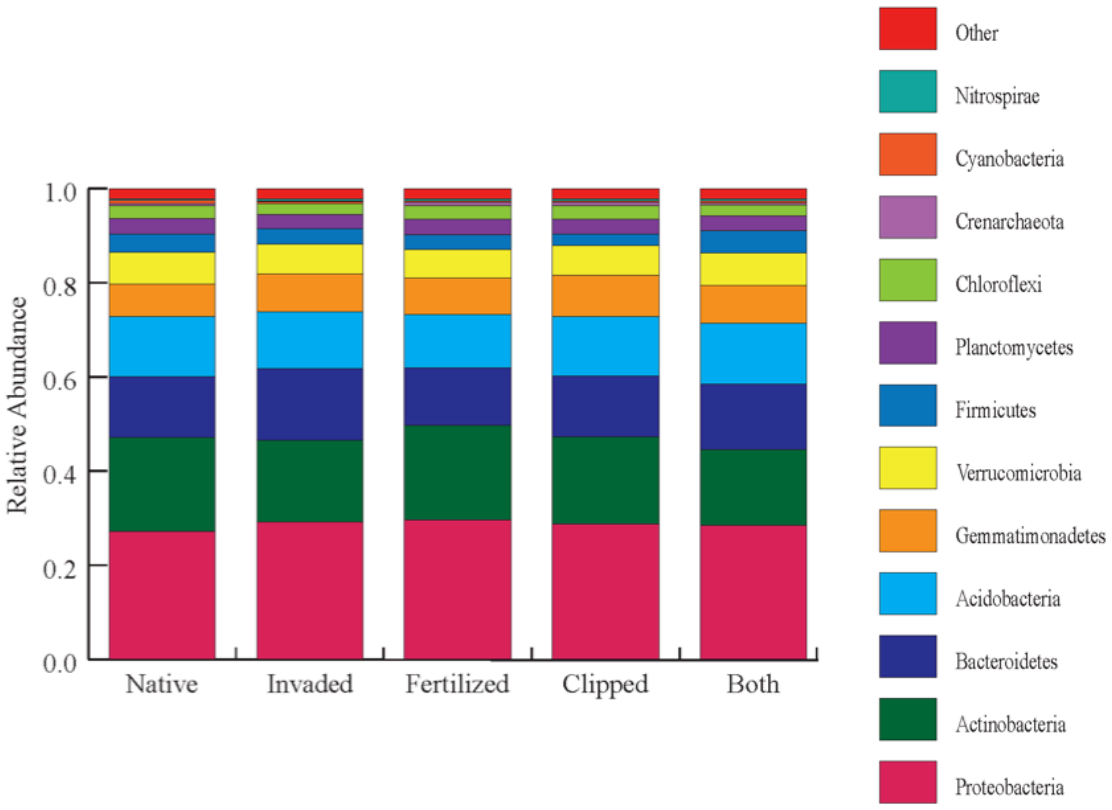

Figure 4-2 Relative abundances (frequency) of the dominant bacterial phyla across treatments. “Other” indicates the combined relative sequence abundance of the additional, rare phyla (28 phyla).

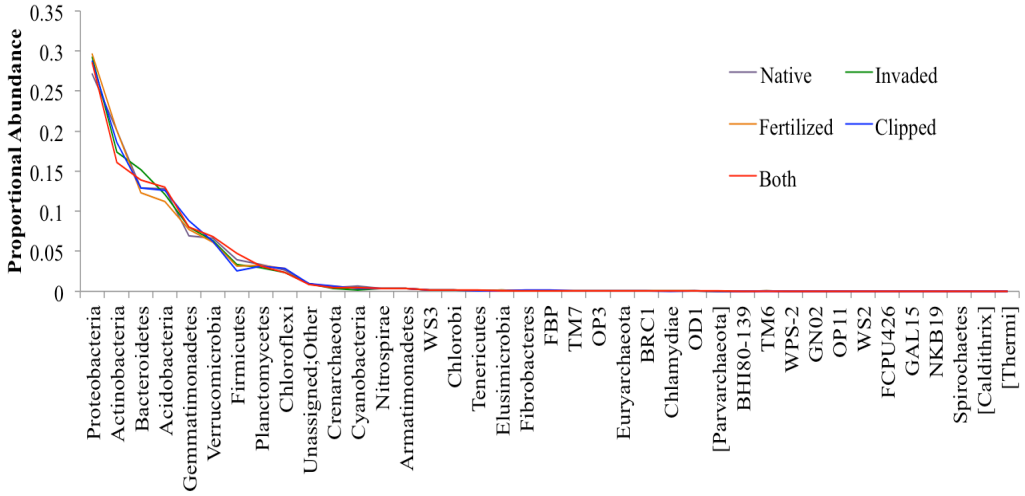

Figure 4-3 Rank abundance curve. Mean proportional abundance of each phylum by treatment.

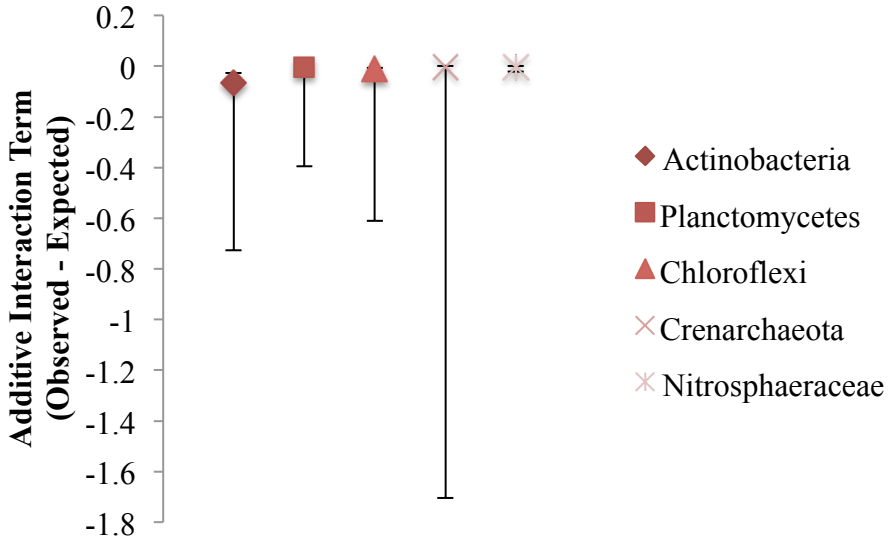

Figure 4-4 Additive interaction term, calculated as observed – expected (mean ± 95 confidence interval), for select taxa that showed significant interaction terms based on a two-way ANOVA. All values fall below zero, suggesting that the interactions between N fertilization and clipping are antagonistic.

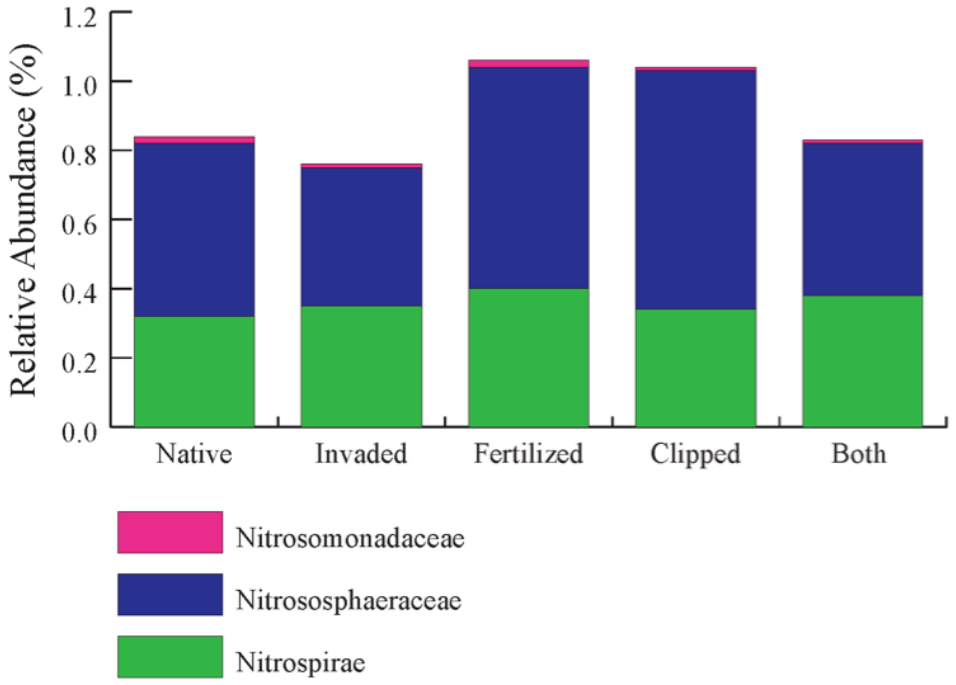

Figure 4-5 Relative abundances (percent) of key taxa involved in nitrification. *Nitrosomondaceae* is a family containing ammonia-oxidizing bacteria, *Nitrososphaeraceaea* is a family containing ammonia-oxidizing archaea, and *Nitrospirae* is a phylum containing nitrite-oxidizing bacteria.

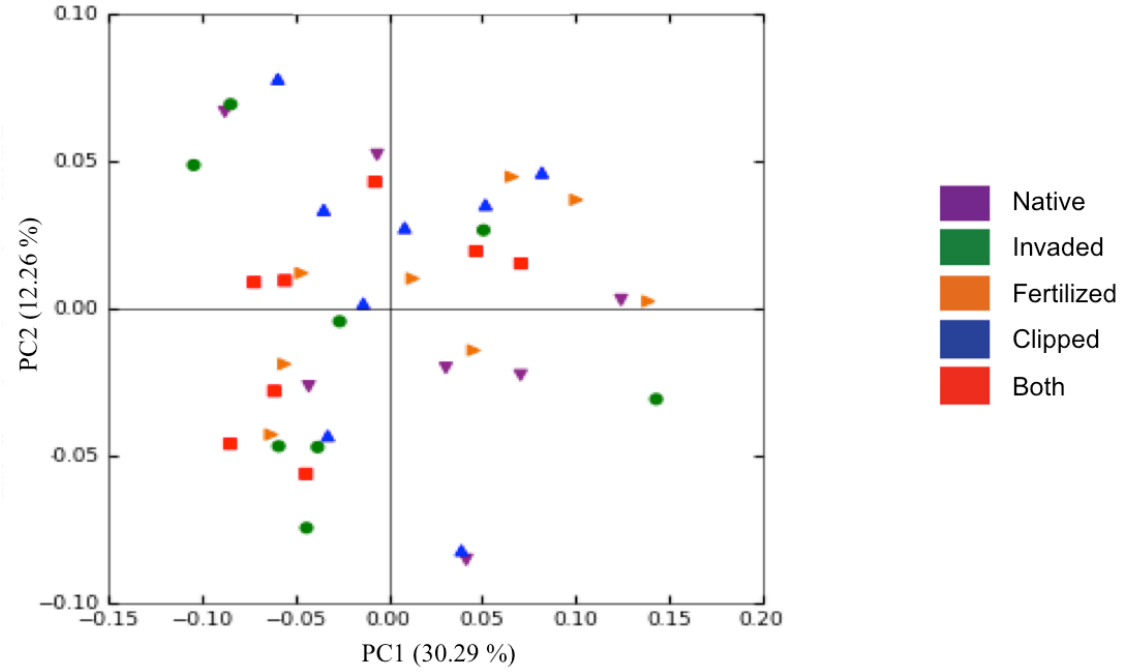

Figure 4-6 Principal coordinate analysis of 16S community composition based on weighted UniFrac distance matrices. Colors are coded by treatment (purple – native community; green – invaded community; orange – fertilized; blue – clipped; red – fertilized x clipped). Together, the first two principle coordinates explain 42.55% of the variation in microbial community composition. Unweighted UniFrac (not shown) demonstrated qualitatively similar patterns.

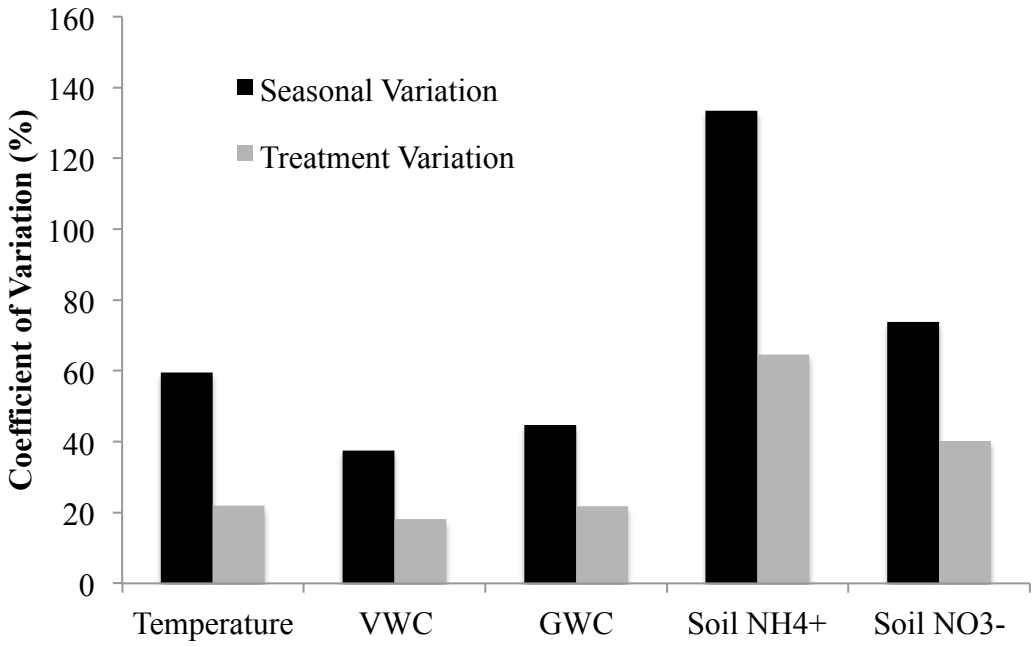

Figure 4-7 Seasonal (black bars) and treatment (gray bars) variation in select soil characteristics. VWC = volumetric water content, GWC = gravimetric water content. Seasonal variation is larger than treatment variation for all displayed characteristics.

5 Conclusion

The overall aim of my dissertation was to quantify the response of soil N cycling to single and combined effects of three principle global change factors affecting Mediterranean grasslands today: exotic plant invasion, aboveground vegetation removal (one component of cattle grazing), and N deposition. Because soil microorganisms largely regulate N cycling, my dissertation also measured the influence of each global change factor on soil microbial community composition, diversity, and the relative abundance of a functional gene (*amoA*) responsible for the first and rate-limiting step of nitrification.

In the first chapter, I found that soils invaded by *A. triuncialis* and *E. caput-medusae* had significantly lower inorganic N availability and potential rates of nitrification and denitrification than native communities, and that reductions were often associated with lower microbial biomass and volumetric soil moisture in surface soil. This observed disparity in N cycling between native and invaded soils could have implications for restoration, given that native perennials are often used to re-vegetate sites after the removal of invasive species; if legacies of altered N cycling persist, the competitive edge may be shifted away from natives and successful re-vegetation may be hindered. In contrast, soil N cycling did not differ between invaded and naturalized plant communities, suggesting that invasion of naturalized grasslands by *A. triuncialis* and *E. caput-medusae* may not induce changes in soil N cycling, and that alterations in N availability are not a mechanism by which these particular exotic species invade.

In the second chapter, invaded grasslands were subjected to factorial replication of aboveground vegetation removal and N fertilization. Cattle grazing and mowing are often used to control invasive plant populations and given that changes in N availability may affect re-vegetation of native plants (as has been observed in other species), it is important to understand how these management tools influence N dynamics of invaded sites. In this case, removal both exacerbated (e.g., N availability and nitrification) and alleviated (e.g., microbial biomass and denitrification) the belowground impacts of *A. triuncialis* and *E. caput-medusae* invasion. Importantly, the response of denitrification potential and active microbial biomass seemed to be dependent upon soil water availability, with wetter years allowing for a larger increase in denitrification potential and microbial biomass.

Not surprisingly, fertilization with NH_4NO_3 consistently increased nitrification potential and soil NO_3^- concentrations of invaded grasslands. However, NH_4^+ concentrations remained unaffected, demonstrating that NH_4^+ is quickly taken up by plants, immobilized by microorganisms, nitrified to NO_3^-, or some combination of these processes. Dramatic increases (443 – 504%) in NO_3^- were observed, suggesting atmospheric deposition of inorganic N could accelerate NO_3^- leaching into groundwater and potentially affect the water quality and ecology of adjacent aquatic ecosystems. This is even more likely given that denitrification potential was concurrently reduced, possibly because of C limitation or reductions in the abundance of fungi that denitrify.

It is important to go beyond measuring ecosystem response to single global change factors and consider whether vegetation removal and N fertilization interact to produce unanticipated effects when co-occurring on the landscape. I hypothesized that vegetation removal and fertilization would interact antagonistically; however, results from this experiment show that they always interact in an additive way and thus their combined effects can be predicted based on single factor studies.

Finally, my second and third chapters show that soil microbial community composition and diversity were unaffected by each global change factor. This observed insensitivity may be due to the large seasonal variability at our site, which is typical of a Mediterranean-type climate. Indeed, seasonal changes in key soil variables such as inorganic N, moisture, and temperature, are much larger than those observed among treatments. Consequently, the resident microbial community may be able to withstand the comparatively minor effects of treatment through malleable metabolic capabilities, gene expression, or mixotrophy. These results have important implications for our understanding of microbial controls on N cycling in Mediterranean grasslands.

Overall, my dissertation demonstrated that microbial community composition was insensitive to exotic plant invasion, aboveground vegetation removal, and N fertilization. However, shifts in total active microbial biomass, N availability, and potential rates of N cycling were observed in response to each treatment. These results challenge the idea that microbial composition, at least as measured by qPCR of the *amoA* gene and next-generation sequencing of 16s rRNA, determines N cycling dynamics; instead they point towards a more nuanced relationship where microbial activity is decoupled from community composition.