

**Non-equilibrium dynamics of ecosystem processes in a  
changing world**

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**Joseph Pignatello Reid**

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**Jacques Finlay**

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# Dedication

For the lovers

## Abstract

The relatively mild and stable climate of the last 10,000 years betrays a history of environmental variability and rapid changes. Humans have recently accelerated global environmental change, ushering in the Anthropocene. Meeting accelerating demands for food, energy, and goods and services has accelerated species extinctions, flows of reactive nitrogen and phosphorus, and warming of the atmosphere. I address the overarching question of how ecosystems will respond to changing and variable environments through several focused studies. Each study examines an ecosystem response to expected environmental changes in the future.

To address how the changing environment affects the sizes and turnover rates of slowly and quickly cycling soil carbon pools, I analyzed the responses of grassland soils to simulated species diversity loss, increased deposition of nitrogen and increased atmospheric CO<sub>2</sub>. I used a soil respiration experiment to fit models of soil carbon pool turnover to respired carbon dioxide. Species diversity, nitrogen deposition and atmospheric CO<sub>2</sub> had no effect on the total soil carbon after 8 years of treatments. Although total soil carbon did not change, the rates of cycling in the fast and slow pools changed in response to elevated CO<sub>2</sub> and diversity loss treatments. Nitrogen treatments increased the size of the slowly cycling carbon pool.

Precipitation variability has increased around most of the world since the industrial revolution. I used plant mesocosms in a greenhouse experiment to manipulate rainfall variability and mycorrhizal associations. I hypothesized that 1) rewetting events result in higher nitrogen fluxes from dry soils than moist soils, 2) a repeated pattern of events caused by low-frequency simulated rainfall results in higher nitrogen fluxes and 3) the better ability of ectomycorrhizal fungi relative to arbuscular mycorrhizal fungi to decompose and assimilate organic nitrogen reduces leaching losses of nitrogen caused by both rewetting events and patterns of repeated events. In response to individual rewetting events, drier soils released more nitrate and total nitrogen than wetter soils. Ectomycorrhizal treatments slightly reduced the effect of antecedent soil moisture on total nitrogen and nitrate losses from rewetting events. This supports my hypotheses

that drier soils release more nitrogen after rainfall events and that ectomycorrhizal associations can reduce nitrogen losses associated with soil rewetting events. However, only ammonium increased in proportion to the variance in rainfall quantity and mycorrhizal treatments had no effect, largely refuting my hypothesis that soils would release more nitrogen when exposed to higher variability patterns of rainfall.

The current pressures that humans place on the environment are only expected to increase as populations and incomes continue to climb. The more than 9 billion people expected on the planet by 2050 require food, energy, shelter and other goods and services. Historically, producing those benefits has resulted in environmental damage, especially nitrogen pollution through agricultural fertilizers, atmospheric nitrogen deposition and human waste. I developed a model to test the effectiveness of various technologies and strategies to reduce the environmental harms associated with meeting the needs of human well-being. I tested the effects of increased crop yields through genetic gains, increased nutrient efficiency in agricultural systems, reduced meat consumption, reduced food waste and improved wastewater treatment on nitrogen yield. The tested levers were mildly effective at reducing nitrogen yield from the baseline business as usual (BAU) scenario, but still resulted in at least 15% greater nitrogen yield than the present. Applied in combination, in the 'Super Ag' scenario, the levers outperformed the sum of their contributions when applied singly. Some levers were more effective in some places than others. Taken together, these results suggest that there is no one solution, and that solutions will be most effective when developed for local conditions and applied in combination.

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# Chapter 1

## Introduction

Earth has never been a steady state. Dramatic environmental changes predate the dawn of life on earth, making non-equilibrium the norm. For example, changes in Earth's spin and orbit around the sun cause periodic swings in Earth's climate, termed Milankovitch cycles and even landscapes evolve through geological processes including erosion and plate tectonics (Zachos et al. 2001). Life itself is one of the largest drivers of environmental change. The great oxygenation event occurred about 2.4 billion years ago when photosynthetic bacteria belched reactive oxygen into the atmospheric, dramatically changing the global environment by oxidizing iron (Holland 2006) and poisoning anaerobic bacteria (Kopp et al. 2005).

Earth has a long history of alteration at the hands of various organisms. Mycorrhizal fungi assisted plants in colonizing land by acquiring and sharing nutrients and water (Heckman et al. 2001), altering terrestrial landscapes and geological processes (Dietrich and Perron 2006). Cyanobacteria and mycorrhizal fungi are two of the many organisms that have dramatically altered the environment of Earth, and now, in the Anthropocene, humans appear to be the dominant driver of environmental change (Crutzen 2002, Steffen et al. 2007). Human societies have presided over a 100 fold increase in species extinction rates, a doubling of reactive nitrogen in the environment, an eight fold increase in phosphorus flows, and at least a 100 ppm increase in atmospheric CO<sub>2</sub> from preindustrial levels (Rockström et al. 2009). The rise in fossil fuel combustion from pre-industrial rates has increased atmospheric CO<sub>2</sub> and atmospheric nitrogen deposition. Higher temperatures associated with climate change increase evapotranspiration

rates and alter the timing and intensity of precipitation (Karl et al. 1995, Easterling 2000, Groisman et al. 2005). These rapid changes in the state of the environment and its variation reinforce the non-equilibrium nature of Earth.

Given the relatively mild environmental conditions over the last 10,000 years, I address the question of how ecosystems will respond to changing and variable environments. In general, there are three classes of responses to change. First, systems can have some level of resistance to change, failing only after reaching some threshold. Second, systems may change in response to environmental drivers, and quickly return to their previous state, that is, they are resilient to changes. Finally, systems can opportunistically take advantage of environmental changes, evolving and changing together with their environment in an anti-fragile manner (*sensu* Taleb 2012, Danchin et al. 2011, see also *creative destruction* in ecosystems Holling 2001).

In Chapter 2 I analyze the responses of grassland soils to experimental species diversity loss, increased deposition of nitrogen and increased atmospheric CO<sub>2</sub>. I address how the shifting environment affects the sizes and turnover rates of slowly and quickly cycling soil carbon pools. I used soil incubations to measure CO<sub>2</sub> respiration rates across the treatments, which I fit to models of soil carbon decay.

Total soil carbon sequestration did not change in response to these environmental drivers, but rates of cycling did change. Elevated CO<sub>2</sub> decreased the residence time of carbon in slowly cycling pools, but did not change pool size. Decreased diversity reduced the size and residence time of fast cycling carbon, but increased the residence time of slowly cycling carbon. Nitrogen additions increased the size of the resistant pool. Carbon sequestration in sandy grassland soils appeared somewhat resistant to changes in elevated CO<sub>2</sub> and species losses.

In Chapter 3 I examine the response of soil nitrogen cycling to precipitation variability. The key questions of this chapter are 1) how does precipitation variability affect nutrient retention in response to single rewetting events and patterns of repeated events? and 2) can mycorrhizal associations reduce the potential for leaching losses in systems affected by precipitation variability? I used plant mesocosms in a greenhouse experiment to experimentally manipulate the variability in precipitation, while keeping overall quantity constant. Cottonwood seedlings were treated with either arbuscular or ectomycorrhizal inoculants prior to the rainfall treatments.

In response to individual rewetting events, drier soils released more nitrate and total nitrogen than wetter soils. Ectomycorrhizal treatments slightly reduced the effect of antecedent soil moisture on total nitrogen and nitrate losses from rewetting events. This supports my hypotheses that drier soils release more nitrogen after rainfall events and that ectomycorrhizal associations can reduce nitrogen losses associated with soil rewetting events. However, only  $\text{NH}_4$  increased in proportion to the cumulative variance in rainfall quantity and mycorrhizal treatments had no effect, largely refuting my hypothesis that soils would release more nitrogen when exposed to higher variability patterns of rainfall. The degree and duration of drying in soils exposed to a repeated pattern of soil drying and rewetting may be more important than the increase in leaching during soil rewetting events. My results suggest that the highest losses of nitrogen will occur when soils dry down enough to limit hydraulic connectivity, but not enough to severely limit microbial nitrification, creating pools of mobile nitrate that readily leach out of soils during rewetting events. These results suggest a degree of resistance in the response of these mesocosms to patterns rainfall variability.

In Chapter 4 I develop and use an integrated global nitrogen balance model to test strategies for reducing the nitrogen pollution associated with providing for human well-being in the year 2050. I asked three key questions about the future of water quality, 1) how might the future compare to the present? 2) where are the places that are most sensitive to changes in scenarios parameters for technologies, mitigation strategies, and population and economic growth? 3) Can these technological and strategic levers help us meet future demands while maintaining or even improving on current water quality? To answer these questions I developed an integrated model of the world's energy, food and economic demands tightly coupled to environmental quality. Unlike other integrated assessment models I developed ours explicitly for the purposes of testing technological and strategic levers for the world in 2050. I tested the effects of increased crop yields through genetic gains, increased nutrient efficiency in agricultural systems, reduced meat consumption, reduced food waste and improved wastewater treatment on nitrogen yield.

The tested levers were mildly effective at reducing nitrogen yield from the baseline business as usual (BAU) scenario. Reducing food waste by half resulted in the largest reductions in nitrogen yield of any single lever of just 2% from BAU. The other levers applied singly were similarly, or less effective at reducing nitrogen yield. Not all levers

were effective everywhere, the largest impacts were in Asia, and the smallest in North America. Applied in combination, in the 'Super Ag' scenario, the levers out performed the sum of their contributions when applied singly. Although the 'Super Ag' scenario decreased global nitrogen yield from BAU by 6.5%, there was still a 15% increase in global nitrogen yield from the present. In this study, the human population growth is the single largest driver of nitrogen pollution. For food and nutrient systems to be most effective they should be developed specific to localities and encourage a diversity of crop choices and nutrient management levers which may convey some degree of anti-fragility to the food system in the face of climate variability.

## Chapter 2

# Biodiversity, nitrogen deposition and CO<sub>2</sub> affect grassland soil carbon cycling but not storage

Short Title: Grassland soil responses to CO<sub>2</sub>, N, diversity

Joseph P. Reid<sup>1</sup>, E. Carol Adair<sup>2</sup>, Sarah E. Hobbie<sup>1</sup>, Peter B. Reich<sup>3</sup>

<sup>1</sup>Department of Ecology, Evolution, and Behavior, University of Minnesota, St Paul, Minnesota 55108, USA

<sup>2</sup>National Center for Ecological Analysis and Synthesis, Santa Barbara, California 93101, USA

<sup>3</sup>Department of Forest Resources, University of Minnesota, St Paul, Minnesota 55108, USA

Correspondence: Joseph P. Reid, tel. +1 612 208 9528, fax +1 612 624 6777, e-mail: jreid@umn.edu

## Abstract

Grasslands are globally widespread and capable of storing large amounts of carbon (C) in soils, and are generally experiencing increasing atmospheric CO<sub>2</sub>, nitrogen (N) deposition and biodiversity losses. To better understand whether grasslands will act as C sources or sinks in the future we measured microbial respiration in long-term laboratory incubations of soils collected from a grassland field experiment after nine years of factorial treatment of atmospheric CO<sub>2</sub>, N deposition and plant species richness on a deep and uniformly sandy soil. We fit microbial soil respiration rates to three-pool models of soil C cycling to separate treatment effects on decomposition and pool sizes of fast, slow and resistant C pools. Elevated CO<sub>2</sub> decreased the mean residence time of slow C pools without affecting their pool size. Decreasing diversity reduced the size and mean residence time of fast C pools (comparing monocultures to plots planted with 16 species), but increased the slow pool mean residence time. Nitrogen additions increased the size of the resistant pool. These effects of CO<sub>2</sub>, N and species richness treatments were largely due to plant biomass differences between the treatments. We found no significant interactions among treatments. These results suggest that C sequestration in sandy grassland soils may not be strongly influenced by elevated CO<sub>2</sub> or species losses. However, high N deposition may increase the amount of resistant C in these grasslands, which could contribute to increased C sequestration.

Keywords: C sequestration, elevated CO<sub>2</sub>, FACE experiment, soil C cycling, biodiversity, nitrogen deposition

*JPR and ECA performed the soil incubations and analyses, and analyzed data. JPR wrote the manuscript with contributions from all co-authors.*



## Introduction

Carbon (C) sequestration is an important ecosystem service that in combination with reduced fossil fuel CO<sub>2</sub> emissions and other measures could reduce already high atmospheric CO<sub>2</sub> levels (Hansen and others 2008). Globally, soils store approximately twice the amount of C as in the atmosphere and terrestrial biomass C pools combined (2344 Pg, 0-3 m, Jobbagy and Jackson 2000), and that C has a mean residence time of 50 years, greater than the mean residence times of either atmospheric (5 years) or terrestrial biomass C pools (9 years, globally averaged, Schlesinger 1997; Scurlock and Hall 1998). Thus, increasing soil C sequestration is one key way to enhance long term C sequestration in terrestrial ecosystems. In particular, grassland soil C has been found to be responsive to increases in atmospheric CO<sub>2</sub>, nitrogen (N) deposition and changes in species diversity (Fornara and Tilman 2008; Jastrow and others 2005; van Groenigen and others 2006). Here, we assess the effects of biodiversity, atmospheric CO<sub>2</sub> concentration, and N deposition on soil C pool sizes and turnover rates using a model grassland system to determine future C sequestration potential.

Elevated CO<sub>2</sub> increases plant biomass contributions to the soil (De Graaff and others 2006; Reich and others 2001a) which can result in modest increases in total soil C (Jastrow and others 2005). However, elevated CO<sub>2</sub> also increases soil microbial biomass and respiration (Craine and others 2001; Dijkstra and others 2005; Gill and others 2006; He and others 2010; Heath and others 2005; Rice and others 1994), reducing root-derived C sequestration (Heath and others 2005). Carbon sequestration is further limited when new C inputs under elevated CO<sub>2</sub> are balanced by losses of old soil C (Adair and others 2009; Gill and others 2002), or contribute only to fast cycling soil pools with little potential for long-term sequestration (Hungate and others 1997; Lichter and others 2005).

Although elevated CO<sub>2</sub> is expected to increase plant production, N availability limits primary productivity in many terrestrial ecosystems (LeBauer and Treseder 2008; Vitousek and Howarth 1991), and can reduce the response of plant productivity to elevated CO<sub>2</sub>, constraining biomass inputs to soils and limiting C sequestration (Reich and others 2006a; Reich and others 2006b). Nitrogen limitation also affects microbial decomposition, further affecting soil C sequestration. Under elevated CO<sub>2</sub>, N-limited

microbes may increase their access to soil N pools by increasing decomposition of soil organic matter to obtain N via the priming effect (Fontaine and others 2004; Fontaine and others 2007). A CO<sub>2</sub>-induced priming effect could reduce soil C sequestration by increasing the turnover rate of soil C, especially in low nutrient soils (but see Dijkstra and others 2005; Fontaine and others 2004). In contrast, relieving N limitation, through N additions in elevated CO<sub>2</sub> environments, could increase plant biomass while maintaining decomposition rates, resulting in soil C sequestration (De Graaff and others 2006; Heath and others 2005; Reich and others 2006a; van Groenigen and others 2006). Nitrogen additions may also increase C sequestration by stabilizing soil C in more resistant fractions (Neff and others 2002).

Although plant species diversity has been widely recognized as an important determinant of ecosystem productivity, its role in determining soil C sequestration remains unclear. High species richness has been shown to increase soil C sequestration by as much as 600% over monocultures (Fornara and Tilman 2008) or to increase sequestration to 2.7 Mg C ha<sup>-1</sup> year<sup>-1</sup> from no net sequestration in monocultures (Tilman and others 2006). However, much of that increase was attributed to the presence and abundance specific species or functional groups (legumes, De Deyn and others 2009; legumes and C4 grasses, Fornara and Tilman 2008), rather than species richness per se. Despite examples of impressive increases in C sequestration, the absolute magnitude of the effect of grassland species richness on soil C sequestration (relative to monocultures) is small and highly variable (Fissore and others 2010). Increasing plant diversity (Tilman and others 2001; Tilman and others 1997), along with elevated CO<sub>2</sub> and N additions (Craine and Jackson 2010) have all been shown to increase plant biomass (Dijkstra and others 2006; Fornara and Tilman 2008), which should result in increased soil C inputs (Adair and others 2009) and thus changes in soil C dynamics.

The majority of research on the effects of elevated CO<sub>2</sub> and N deposition on soil C has considered soil C as a single pool (but see Dijkstra and others 2005; Neff and others 2002). However, because the soil C pool is so large, it is difficult to detect change over short periods of time. Additionally, soil C varies in quality and accessibility to microbes from labile to recalcitrant. To represent this conceptually, models often divide soil C into two or more pools. The fastest cycling pools are typically the smallest and are referred to as active, labile or fast pools. In two pool models there is a second, slower

pool, whereas in three-pool models there is a pool with intermediate turnover time that is referred to as the slow pool, and the slowest pool is referred to as resistant or recalcitrant. We will refer to three-pool soil C models using the fast, slow, and resistant terminology. Few studies have examined how diversity, CO<sub>2</sub> or N treatments influence the sizes and turnover rates of multiple soil pools. This distinction is important because long-term C sequestration depends largely on changes to slow and resistant pools.

Here, we describe a laboratory incubation study of field-collected soils from a large-scale experiment where CO<sub>2</sub>, N and plant diversity were manipulated for nine years. We expand on previous work (Dijkstra and others 2005) by using a three-pool model that provides a more detailed representation of soil dynamics (Paustian and others 1992) and that allowed us to estimate the sizes and dynamics of the slow and resistant pools. We used estimated soil pool sizes and decomposition rates to test several hypotheses about how soil C pools respond to multiple — and likely interacting — global change factors. Elevated CO<sub>2</sub> was hypothesized to increase the decay rates of the fast and slow pools by increasing labile C inputs and priming microbial decomposition (Fontaine and others 2004). We expected N additions to increase the size of the resistant pool and decrease the decay rate of the slow pool by decreasing lignin decomposition (Dijkstra and others 2004) and by reducing priming (Fontaine and others 2004; Pregitzer and others 2008; Zak and others 2008). Nitrogen additions were expected to interact with elevated CO<sub>2</sub> to increase total soil C by reducing nutrient limitation of primary production (increasing soil inputs) and microbial respiration (decreasing priming losses, Fontaine and others 2004). High plant diversity was hypothesized to increase the decay rate of the fast pool by increasing microbial metabolism through increased plant biomass and C inputs to soils (Zak and others 2003). Finally, we hypothesized that elevated CO<sub>2</sub>, N and plant diversity treatments would increase biomass and therefore increase total soil organic C.

## Methods

### BioCON

This research was conducted within the Biodiversity, CO<sub>2</sub>, and N experiment (BioCON, Reich and others 2001a; Reich and others 2001b), which was established in 1997 in a nearly level old field in the Cedar Creek Ecosystem Science Reserve (CCESR),

Minnesota, USA (Lat.  $45^{\circ}N$ , Long.  $93^{\circ}W$ ). Soils in this area are very homogeneous, sandy, and nutrient poor (Typic Udipsamments on the Anoka sand plain, Grigal and others 1974). Mean annual precipitation is 660 mm with mean monthly temperatures of  $-11^{\circ}C$  in January and  $22^{\circ}C$  in July.

In 1997 the vegetation from six 20 m diameter circular areas was removed. Soil was tilled uniformly to a depth of 25cm and fumigated with methyl bromide to eliminate the soil seed bank. Soils were reinnoculated with microbes from surrounding old field soils. By 2000, arbuscular mycorrhizal fungal communities and soil respiration had recovered to levels similar to surrounding old field areas (Wolf and others 2003 unpublished soil C flux data). In June of 1997, 296 2x2 m plots were seeded with 1, 4, 9, or 16 grassland species, randomly chosen from 16 species in four functional groups (C3 and C4 perennial grasses, non-legume forbs, and legumes) at a rate of  $12\text{ g m}^{-2}$ , with seed mass divided evenly among the species in a plot. The 16 species used were all native or naturalized to the CCEsr: the C4 grasses *Andropogon gerardii* Vitman, *Bouteloua gracilis*, *Schizachyrium scoparium* (Michaux) Nash, and *Sorghastrum nutans* (L.) Nash; the C3 grasses *Agropyron repens* (L.) Beauv., *Bromus inermis* Leysser, *Koeleria cristata* Pers., and *Poa pratensis* L.; the forbs *Achillea millefolium* L., *Anemone cylindrica* A. Gray, *Asclepias tuberosa* L., and *Solidago rigida* L.; and the legumes *Amorpha canescens* Pursh, *Lespedeza capitata* Michaux, *Lupinus perennis* L., and *Petalostemum villosum* Nutt. Plots were irrigated during the 1997 growing season, but not in subsequent years.

Plots in three of the six rings have been treated with ambient +180 ppm  $\text{CO}_2$  during each growing season since 1998 (using FACE technology). Beginning in 1998, half of the plots were fertilized with  $4\text{ g N m}^{-2}\text{ yr}^{-1}$  applied in three doses during the year (May, June, and July) as slow release  $\text{NH}_4\text{NO}_3$ . All plots were burned two of every three years (2000, 2002, 2003, and 2005), a common management practice that mimics natural fire frequencies in tall grass prairies.

The BioCON main experiment is a split-plot arrangement of treatments in a completely randomized design. The  $\text{CO}_2$  treatment is the whole-plot factor. The subplot treatments of species richness and N addition were randomly distributed and replicated in individual plots among the six rings. For this research, we utilized all of the 16 species plots and 8 of the 16 monoculture treatments, 2 from each functional group: C4 grasses *Andropogon gerardii* Vitman and *Sorghastrum nutans* (L.) Nash; the C3 grasses

*Agropyron repens* (L.) Beauv. and *Bromus inermis* Leysser; the forbs *Asclepias tuberosa* L., and *Solidago rigida* L.; and the legumes *Amorpha canescens* Pursh and *Lespedeza capitata* Michaux. We limited this experiment to just the monocultures and 16 species plots to capture the largest possible differences in belowground biomass and to keep the experiment a manageable size.

In August of 2006, we sampled soils from the 48 16 species plots and 64 monoculture plots (total of 112 plots) by taking three 2.5 cm diameter cores (0-20 cm) per plot. Soils were composited by plot and immediately sieved (2 mm). Visible roots were removed by hand. We took immediate subsamples for soil C respiration incubations and gravimetric soil water content. The remaining soil was air dried, ground, and subsampled for total C and N and non-hydrolyzable C and N analyses.

## Soil analyses

Resistant soil C was estimated using an acid digest procedure that hydrolyzes polysaccharides and nitrogenous material, leaving a residue consisting primarily of lignin and polyaromatic humics (Martel and Paul 1974; Sollins and others 1999). Identifiable plant materials were removed from air-dried, ground soil. One-gram soil samples were refluxed for 16 hours in digestion tubes with 10 mL of 6 M hydrochloric acid solution. The remaining residue was filtered, washed with 100 mL of nanopure water, dried for 24 hours in a 60 °C oven, weighed, and analyzed for total C by combustion (Model ECS 4010, COSTECH Analytical, Valencia, CA). The remaining nonhydrolyzable, or chemically resistant, C represents resistant soil C, which <sup>14</sup>C-dating indicates is much older than bulk soil (Paul and others 2006). A subsample of the dried and ground whole soil was also analyzed for total organic C by dry combustion (as above).

To quantify organic soil C pools and decomposition rates we placed 20 g of moist soil into 120 mL polyethylene specimen cups and brought soils to a common moisture content (70% field capacity to prevent rapid drying in these sandy soils (Dijkstra and others 2006)) using nanopure water to ensure that no additional nutrients were added. Specimen cups were placed in 1-L glass jars and were incubated aerobically in the dark at a constant temperature (21 °C) for 391 days. On each sampling day (1, 4, 7, 13, 27, 46, 74, 152, 168, 222, 273, 324, and 391 d), we sampled CO<sub>2</sub> production over 24 hours. On each date, jars were capped and the headspace was sampled immediately

through a septum in the lid. Headspace was sampled again after 24 hours. Headspace samples were immediately analyzed for CO<sub>2</sub> on a gas chromatograph (Shimadzu GC14, Shimadzu Scientific Instruments, Wood Dale, IL) using a thermal conductivity detector and a Poropak N column. Daily soil C respiration rates were calculated by determining the difference between CO<sub>2</sub> concentrations in the initial (time = 0) and final (time = 24 hr) samples. Between sampling periods, jars were covered with a polyethylene film to allow O<sub>2</sub> exchange and minimize soil water loss.

Fitting one, two, or three pool models to soil incubation respiration data allows for the analytical estimation of soil C pools and fluxes. Either cumulative respiration or daily respiration rate data may be used for model fitting. Several authors have suggested that using cumulative respiration data accumulates errors while dampening noise and providing a false sense of security in the form of high  $r^2$  values (Alvarez and Alvarez 2000; Ellert and Bettany 1988; Hess and Schmidt 1995). Thus, to avoid autocorrelation in residuals and dependence among data points (Hess and Schmidt 1995), we fit all models to daily respiration rates.

Because incubation data alone are not sufficient to analytically estimate the size and flux of resistant C, we used the nonhydrolyzable C fraction as an estimate of the resistant pool, and fit a three pool model to the daily respiration rates (Paul and others 2006; Paul and others 1999; Pendall and King 2007):

$$C_{rate}(t) = k_f(C_f e^{-k_f t}) + k_s[(C_t - C_f - C_{NHC})e^{-k_s t}] + k_r(C_{NHC} e^{-k_r t})$$

where  $C_{rate}$  is the daily respiration rate (mg C g soil<sup>-1</sup> day<sup>-1</sup>),  $C_f$  is the labile C pool (mg C g soil<sup>-1</sup>),  $C_{NHC}$  is nonhydrolyzable or resistant C (NHC; mg C g soil<sup>-1</sup>),  $C_t$  is total C (mg C g soil<sup>-1</sup>),  $k_f$ ,  $k_s$ , and  $k_r$  are the decomposition rates of the labile (fast), slow, and resistant pools (respectively; day<sup>-1</sup>), and  $t$  is time in days. The slow C pool ( $C_s$ ) is defined in the above equation as  $C_t$  minus the sum of  $C_f$  and  $C_{NHC}$ . The mean residence time (MRT) of the resistant C pool was constrained to be > 1000 years (Paul and others 2006; Pendall and King 2007  $k_r = 2.7 \times 10^{-6}$  day<sup>-1</sup>). As was found by Paul et al. (2001) and Pendall and King (2007), the choice of a  $k_r$  (100 – 1000 years) did not influence the parameter estimates of the faster soil C pools or fluxes (Appendix A).

Although we fit one, two, and three pool models to the daily respiration data from

each soil incubation jar (Appendix A), we chose to focus on the parameter estimates from the three pool model for several reasons: (1) the two and three pool models better accounted for the long term dynamics of soil respiration in our incubations than did the single pool model; (2) the two and three pool models fit the data equally well (Appendix A); (3) obtaining separate estimates for slow and resistant pools and fluxes allowed us to parse out the effects of BioCON treatments on each pool (versus a two pool model which lumps resistant and slow C into the second, slow pool) and expand upon previous work in BioCON that analyzed long-term incubations using a two-pool model (2005); and (4) Paul et al. (2001) found that using a two pool model (constrained by total C) substantially underestimated both the size and decomposition rate of the slow C pool. We also found that the two pool model consistently resulted in a slower  $k_s$ , but a larger  $C_s$  (Appendix A). Estimates of  $k_f$  and  $C_f$  were unaffected by model choice (linear regressions of parameter estimates from both models had intercepts  $\sim 0$  and slopes  $\sim 1$ ; Appendix A). The three pool model explained more than 90% of the variation in daily respiration rates in 66/112 cases; between 70-90% of the variation in the data in 42/112 cases; and between 60-70% of the variation in the data in 4 cases (Appendix A).

## Data Analysis

We performed several different analyses to investigate the effects of N, CO<sub>2</sub>, and species richness on soil C pools and fluxes. Total soil C and nonhydrolyzable C were analyzed using an ANOVA with ring nested within CO<sub>2</sub> treatment as a random effect. All treatments were considered fixed effects. The same ANOVA was performed on the soil C pool and flux parameter estimates from the three pool model:  $k_f$ ,  $k_s$ ,  $C_f$ , and  $C_s$ . We used an ANCOVA with total plant biomass in each plot averaged over the experiment duration (1998-2006) as a covariate in our mixed-effects model to determine the degree to which plant biomass was responsible for treatment effects (JMP 9.0.1, SAS Institute, Cary, NC). We also used a similar ANCOVA with below-ground plant biomass in each plot averaged over the experiment duration, but the results were nearly identical to the total plant biomass results (Appendix B), thus we focused on the results for total plant biomass. Dependent variables with non-normal residuals (Shapiro-Wilkes test for normality) were natural log transformed ( $k_f$ ,  $k_s$ ,  $C_f$ , and  $C_s$ ) to meet normality

assumptions.

## Results

Averaged across all treatments, total soil C was 6.03 mg C g soil<sup>-1</sup>, fast or labile C was 0.14 mg C g soil<sup>-1</sup> or 2% of total soil C, slow C was 4.02 mg C g soil<sup>-1</sup> or 67% of total soil C, and resistant C (NHC) was 1.87 mg C g soil<sup>-1</sup> or 31% of total soil C. The mean residence time (MRT) of fast and slow C averaged 19 days ( $k_f = 18.6 \text{ yr}^{-1}$  or  $0.051 \text{ day}^{-1}$ ) and 9 years ( $k_s = 0.10 \text{ yr}^{-1}$  or  $0.00027 \text{ day}^{-1}$ ), respectively.

Contrary to our expectations, total soil C did not change significantly in response to CO<sub>2</sub>, diversity or N or their interactions (Figure 2.1). Although total C storage did not change with treatments, the distribution of C among fast, slow and resistant pools did.

High species richness nearly doubled the fast pool size ( $C_f$ ) and decreased the decay rate of the fast pool by 39% compared to monocultures (Figure 2.2). High species richness also increased the decay rate of the slow pool by 27% compared to monocultures. There was no concurrent change in slow pool size between species richness treatments, indicating that monoculture plots had lower inputs to the slow pool to match the lower observed decay rates.

In response to elevated CO<sub>2</sub> we observed a marginally significant 22% increase in the slow pool decay rate. There was no concurrent change in slow pool size in elevated CO<sub>2</sub> plots, indicating that inputs to the slow pool under elevated CO<sub>2</sub> were increased at a rate that roughly matched the observed decay rate (Figure 2.3).

Although N additions had no significant effect on fast and slow pool sizes or decomposition rates, we observed a 10% increase in the size of the resistant C pool (NHC) in the N addition treatment compared to the ambient treatment, although this effect was only marginally significant (Figure 2.4).

In contrast to our hypothesis that elevated CO<sub>2</sub> would increase soil C in the presence of sufficient N (N additions), there were no significant interactions between CO<sub>2</sub>, N and species richness (Table 2.1).

Because higher levels of all three treatments increased plant biomass (Reich and others 2006b), we further analyzed our results with the total plant biomass in each plot



averaged over the experiment duration (1998-2006) as a covariate in our mixed-effects model. Total plant biomass accounted for all of the effects of CO<sub>2</sub>, N and diversity, except on the slow pool decay rate (ANCOVAs, Table 2.2). Accounting for total plant biomass reversed the effect of diversity on  $k_s$ . The slow pool decay rate increased by 43% in single species treatments ( $k_s = 0.116 \text{ yr}^{-1}$  or  $0.00032 \text{ day}^{-1}$ , MRT 8.6 years) compared to 16 species treatments ( $k_s = 0.081 \text{ yr}^{-1}$  or  $0.00022 \text{ day}^{-1}$ , MRT 12.3 years; ANCOVA, *diversity*:  $P = 0.0244$ ).

## Discussion

Our results suggest that grasslands on coarse-textured soils subjected to increasing atmospheric CO<sub>2</sub>, N deposition and species losses may not become strong C sinks. Although we found no significant change in total soil organic C after nine years of treatments, we found significant effects of species diversity on fast pool size and decay rate; of diversity and CO<sub>2</sub> on slow pool cycling; and of N on resistant pool size. Changes to the fast pool size and decay rate caused by loss of species richness are unlikely to result in substantial loss of C sequestration potential because of the small size of the fast pool (2% of total soil organic C) and its short mean residence time (19 days). Elevated CO<sub>2</sub> and increasing diversity both increased the rate of C cycling in the slow pool without affecting its size. Finally, although the effect of N additions on the resistant pool was small (10% increase in resistant C which is 31% of total soil organic C) our results suggest that N additions could slowly increase long-term C storage.

## Biodiversity

While the effect of biodiversity on productivity is relatively well understood (Cardinale and others 2006; Reich and others 2001b), few studies have examined the effects of species richness on soil C (but see Fornara and Tilman 2008; Tilman and others 2006), and even fewer have investigated its effects on different soil C pools and fluxes (but see Dijkstra and others 2005). In our study, high species richness resulted in a larger fast-cycling pool (consistent with results reported for fast-cycling pools in four species versus monoculture treatments Dijkstra and others 2005) that decomposed at a slower

rate. The results of the ANCOVA suggest that these species richness effects were due to greater plant biomass in the high richness treatments (as also concluded by Dijkstra and others 2005). Despite the increase in total plant biomass, there was no stimulation of old or resistant C decomposition as previously reported at this site (Dijkstra and others 2006), and also no offset of soil respiration by increased litter inputs associated with N additions in high species-richness plots (Dijkstra and others 2005).

Increasing species richness decreased the fast pool decay rate, a change associated with higher total plant biomass in more diverse plots. Increasing species richness also increased the slow pool decay rate without affecting its size — suggesting an associated increase in slow pool inputs and accelerated cycling of slow soil C. Increased slow-pool C cycling could be due to an increase in slow C inputs under non-limiting N conditions for microbes (Kaye and Hart 1997; Kuzyakov 2002), changes in the quality of slow C inputs (e.g., the ratio of root litter to exudates or decreased C/N of root litter and exudates), or the rhizosphere priming effect (Fontaine and others 2004; Pregitzer and others 2008; Zak and others 2008). High root C inputs should increase slow C decomposition when there is sufficient N available for microbes or if the inputs are of higher quality (e.g., lower C/N). The rhizosphere priming effect would cause an increase in decomposition of older, N-rich soil C when N supply to microbes is insufficient. Unfortunately, our results do not allow us to reject any of the potential causes of increased slow-pool C cycling. However, there is evidence that the increase in total belowground C allocation in diverse plots is due to root biomass — suggesting a higher ratio of root tissues to exudates in diverse plots relative to monocultures (Adair and others 2009) — hinting that changes in slow pool inputs may be driving the increased slow-pool cycling.

Our results contrast those of similar research at Cedar Creek Ecosystem Science Reserve (Fornara and Tilman 2008; Tilman and others 2006) that found significantly higher soil C accumulation in 16 species plots compared to monocultures at the same depths that we sampled. In that study, the topsoil was removed from the plots prior to the start of their experiment. Thus, initial soil C concentrations were lower, which may have contributed to the higher rates of total C accumulation in diverse plots in that study compared to in our study.

## Nitrogen

Soil C dynamics have been shown to depend heavily on the available N in soils (DeForest and others 2004; Fontaine and others 2003; Fontaine and others 2004; Fontaine and others 2011; Pregitzer and others 2008), with old soil C acting as a nutrient bank (Sensu Fontaine and others 2011). Consistent with this mechanism, N additions in this low-nutrient grassland have stimulated cellulose decomposition (Keeler and others 2009) and increased the decomposition rate of labile C in the soil (Dijkstra and others 2005; Dijkstra and others 2006) and litter (Hobbie and others in revision) probably due to the alleviation of N limitation of C decomposition. Nitrogen additions increased resistant C by 10% in our three-pool model of soil C, with no changes to the slow pool size or rate. As hypothesized, this increase was associated with higher total plant biomass. Our results are consistent with the nutrient bank hypothesis (Sensu Fontaine and others 2011), though we can not differentiate between the possible mechanisms of physio-chemical stabilization (e.g. stabilization of lignin rich litter inputs by N additions, Dijkstra and others 2004; Von Lutzow and others 2008) that were responsible for the marginally significant increase in resistant C that we observed in the presence of N additions.

Because the effect of N additions on the resistant pool was small and the resistant pool is less than one-third of total SOC, there was no detectable change in total SOC. However, the resistant pool has an assumed mean residence time of 1000 years, so even a modest increase of 10% could result in a long-term increase in C sequestration. The projected increase in total SOC in N addition treatments compared to ambient N treatments would be 5% after 13 years and 20% after 49 years.

## Elevated CO<sub>2</sub>

As expected, elevated CO<sub>2</sub> alone did not increase C storage, and actually led to increased turnover of slow C which taken alone would result in decreased C storage over the long-term. However, the slow pool size remained constant between ambient and elevated CO<sub>2</sub>, indicating a concurrent increase in inputs to the slow pool in the elevated CO<sub>2</sub> treatments. Our results therefore contrast with the decline in sequestration associated with elevated CO<sub>2</sub> reported by Heath and others (Heath and others

2005), but provide further support for increased rates of belowground C cycling under elevated CO<sub>2</sub> (Adair and others 2009; Hagedorn and others 2003; Hungate and others 1997; Van Kessel and others 2000). The implied increase in C inputs is consistent with increased plant biomass observed in response to elevated CO<sub>2</sub> in grasslands (Adair and others 2009; Dijkstra and others 2005; Dijkstra and others 2006; Reich and others 2006a; Reich and others 2006b). The higher decay rate of the slow pool in elevated CO<sub>2</sub> was associated with increases in plant biomass at elevated CO<sub>2</sub>. Consistent with the soil nutrient bank hypothesis (Sensu Fontaine and others 2011), and the progressive N limitation hypothesis, our results suggest that additional plant production in elevated CO<sub>2</sub> may increase soil C:N, causing N-limited microbes to increase decomposition of slow C for access to N (Gill and others 2002). Using a three-pool model of soil C turnover we were able to detect faster C cycling and higher C inputs in the slow pool that others were not able to detect with a simpler two-pool model (Dijkstra and others 2005).

## Interactions

Contrary to our expectations, we found no significant interactions between CO<sub>2</sub> and N on any soil C pools or turnover rates. Interestingly, our results suggest that CO<sub>2</sub> and N affect belowground C cycling in different ways: CO<sub>2</sub> increased the inputs to the slow pool and its decomposition rate, whereas N increased the resistant pool size. The differences in effects are likely due to differences in total belowground carbon allocation (TBCA). Adair and others (2009) found that both CO<sub>2</sub> and N increased TBCA at BioCON. Although the effect of N was due entirely to concurrent increases in root biomass, the effects of CO<sub>2</sub> on TBCA could not be explained by root biomass alone (Adair and others 2009), implying an increase in allocation to root exudates or arbuscular mycorrhizae (AM), consistent with studies elsewhere (Pendall and others 2004; Treseder and Allen 2000; Treseder 2004). Increased allocation to mycorrhizae at BioCON is supported by increased AM spore volume in elevated CO<sub>2</sub> plots (Antoninka and others 2011; Wolf and others 2003). An increase in either root exudates or allocation to AM caused by elevated CO<sub>2</sub> is likely to prime decomposition of older, N-rich soil C to alleviate N limitation (Fontaine and others 2011; the microbial activation hypothesis, Kuzyakov 2002). In contrast, the N additions resulted in allocation of TBCA to root biomass

(Adair and others 2009). The lack of additional C inputs to root exudates or AM in elevated N treatments limits mycorrhizal exploration and the decomposition of older, N-rich soil C, possibly preserving slow and resistant soil C. Thus, we found CO<sub>2</sub> and N treatment effects to be additive and not interactive.

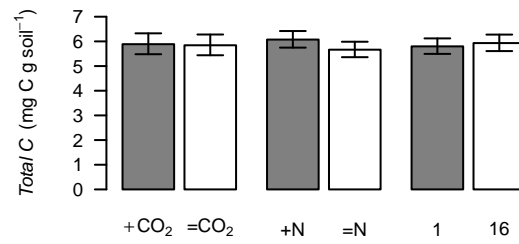
## Conclusions

Our results suggest that while elevated CO<sub>2</sub>, added N and changes in diversity alter belowground C cycling, these changes are unlikely to result in rapid, substantial C sequestration in coarse textured soils such as studied here. Elevated CO<sub>2</sub> only increased the cycling rate of slow C, suggesting that a portion of the previously observed increases in rates of belowground cycling at elevated CO<sub>2</sub> (Adair and others 2009; Hungate and others 1997) is likely associated with slowly cycling soil pools of C (in the absence of concurrent labile plant C inputs). Increasing species richness also increased belowground cycling of slow C, but also resulted in larger, more slowly decaying fast C pools; changes that are unlikely to increase total C storage. We believe that the removal of all topsoil in previous studies of the effects of species diversity on soil C storage (Fornara and Tilman 2008; Tilman and others 2006) may explain the large soil C increases that others have observed that we were unable to replicate. Although CO<sub>2</sub> and species richness treatments are unlikely to increase soil C storage, our results suggest that N additions to N-limited grasslands on coarse textured soils may result in a small, long-term sink for soil C.

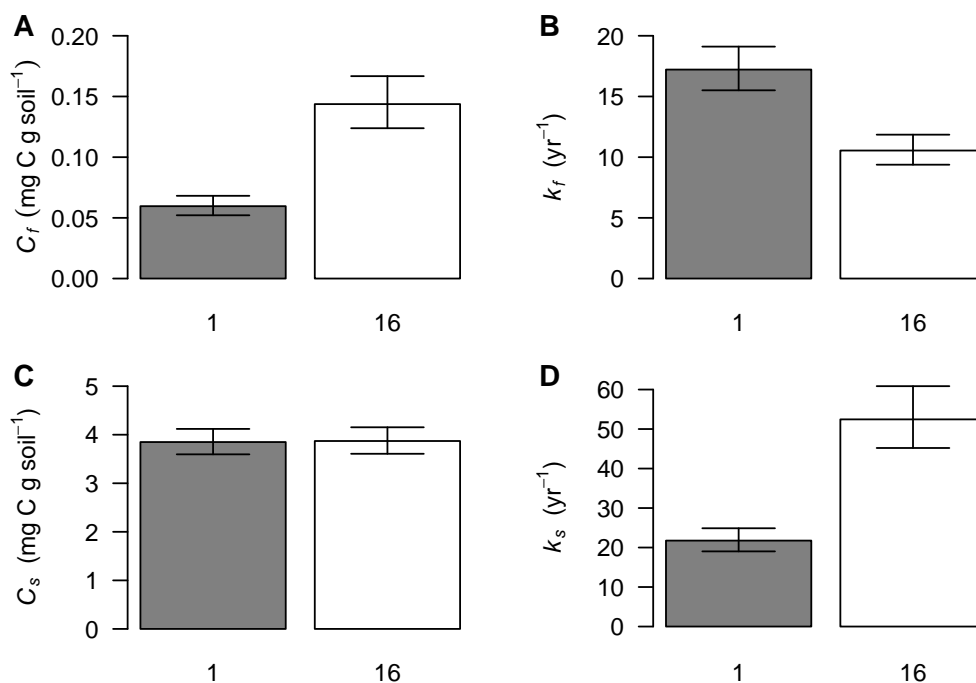
## Acknowledgements

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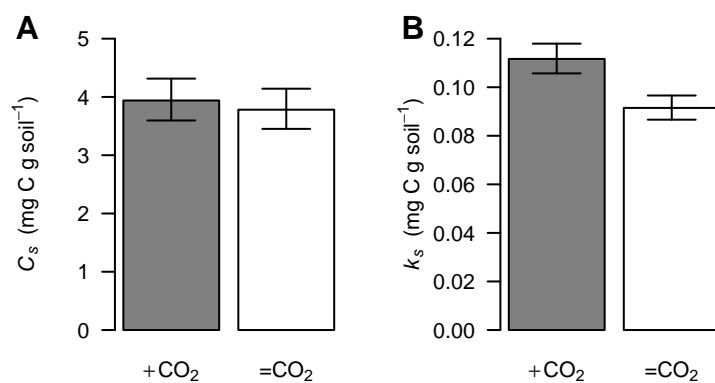
## Figures and Tables



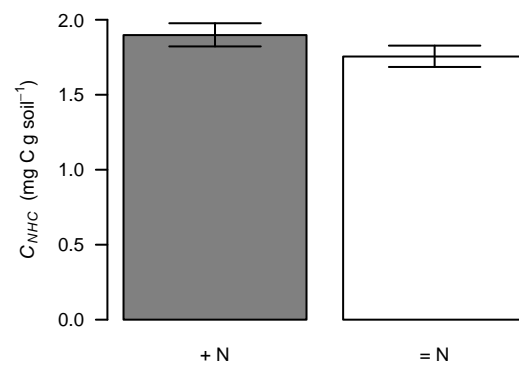
**Figure 2.1** Least squares means  $\pm$  standard error of total C for all treatments. In the ANOVAs there were no significant treatment effects.



**Figure 2.2** Least squares means  $\pm$  standard errors of the estimated (a) fast C pool size (mg C g soil<sup>-1</sup>), (b) fast pool decomposition rate (yr<sup>-1</sup>), (c) slow C pool size (mg C g soil<sup>-1</sup>), and (d) slow pool decomposition rate (yr<sup>-1</sup>) in monocultures and 16 species plots. In the ANOVAs, species richness significantly increased fast pool size, but decreased the fast decomposition rate. Species richness had no significant effect on the size of the slow pool.



**Figure 2.3** Least squares means  $\pm$  standard errors of the estimated (a) slow C size (mg C g soil<sup>-1</sup>) and (b) decomposition rate (yr<sup>-1</sup>) in soils from the elevated and ambient CO<sub>2</sub> treatments. In the ANOVA, elevated CO<sub>2</sub> tended to increase the slow decomposition rate.



**Figure 2.4** Least squares means  $\pm$  standard errors of the resistant C size ( $\text{mg C g soil}^{-1}$ ) in soils from the elevated and ambient N treatments. In the ANOVA, elevated N tended to increase the resistant C pool size.



**Table 2.1** ANOVA results for three pool model

		$N$	$C_f$	$k_f$	$C_s$	$k_s$	$C_{NHC}$	$Total\ C$
CO <sub>2</sub>	ambient	56	0.089	0.033	3.781	0.00025†	1.9	5.8
	elevated	56	0.096	0.041	3.939	0.00031†	1.8	5.9
Nitrogen	ambient	56	0.086	0.035	3.761	0.00027	1.8†	5.7
	elevated	56	0.099	0.039	3.961	0.00028	1.9†	6.1
Species number	1	64	0.060**	0.047**	3.849	0.00025**	1.8	5.8
	16	48	0.144**	0.029**	3.870	0.00031**	1.8	5.9
$R^2$			0.25	0.17	0.23	0.10	0.15	0.21

Mixed effects model parameter estimates of fast and slow C pool sizes ( $C_f$ ,  $C_s$ ,  $C_{NHC}$  and  $Total\ C$ : mg C g soil<sup>-1</sup>), and decomposition rates ( $k_f$  and  $k_s$ : day<sup>-1</sup>). † $P \leq 0.1$ , \* $P \leq 0.05$ , \*\* $P \leq 0.01$  (ANOVA). No significant interactions were found  $P \leq 0.1$ .

**Table 2.2** ANCOVA results for three pool model

		$N$	$C_f$	$k_f$	$C_s$	$k_s$	$C_{NHC}$	$Total\ C$
CO <sub>2</sub>	ambient	56	0.090	0.033	3.78	0.00025	0.19	5.864
	elevated	56	0.082	0.043	3.95	0.00028	0.17	5.844
N	ambient	56	0.094	0.034	3.75	0.00029	0.18	5.676
	elevated	56	0.079	0.042	3.98	0.00025	0.19	6.037
Species number	1	64	0.093	0.04	3.81	0.00032*	0.19	5.868
	16	48	0.079	0.036	3.92	0.00022*	0.18	5.840
Total biomass		112	0.00153**	-0.000545	-0.000036	0.00087**	0.00011	0.0000403
$R^2$			0.34	0.18	0.23	0.25	0.15	0.21

Mixed effects model parameter estimates of fast and slow C pool sizes ( $C_f$ ,  $C_s$ ,  $C_{NHC}$  and  $Total\ C$ : mg C g soil<sup>-1</sup>), and decomposition rates ( $k_f$  and  $k_s$ : day<sup>-1</sup>). † $P \leq 0.1$ , \* $P \leq 0.05$ , \*\* $P \leq 0.01$  (ANCOVA). No significant interactions were found  $P \leq 0.1$ .

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## Chapter 3

# Rainfall variability and mycorrhizal associations affect nitrogen retention in tree mesocosms

Joseph Pignatello Reid

Department of Ecology, Evolution and Behavior, University of Minnesota – Twin  
Cities, St. Paul, MN 55108

jreid@umn.edu

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## Abstract

In this study I address how changing precipitation patterns affect nitrogen retention through mycorrhizal symbioses in a mesocosm study. I hypothesized that 1) rewetting events result in higher leaching nitrogen fluxes from dry soils than moist soils, 2) a repeated pattern of events caused by low-frequency simulated rainfall results in higher nitrogen fluxes and 3) the better ability of ectomycorrhizal fungi relative to arbuscular mycorrhizal fungi to decompose and assimilate organic nitrogen reduces leaching losses of nitrogen caused by both rewetting events and patterns of repeated events. I measured the effects of rainfall variability and mycorrhizal type by measuring nutrients in leachate from 37 cottonwood (*Populus deltoides*) seedling mesocosms with either arbuscular or ectomycorrhizal associations watered under low or high frequency watering regimes for 12 weeks.

In response to individual rewetting events, drier soils released more nitrate and total nitrogen than wetter soils. Ectomycorrhizal treatments slightly reduced the effect of antecedent soil moisture on total nitrogen and nitrate losses from rewetting events. This supports my hypotheses that drier soils release more nitrogen after rainfall events and that ectomycorrhizal associations can reduce nitrogen losses associated with soil rewetting events. However, only  $\text{NH}_4$  increased in proportion to the cumulative variance in rainfall quantity and mycorrhizal treatments had no effect, largely refuting my hypothesis that soils would release more nitrogen when exposed to higher variability patterns of rainfall. The degree and duration of drying in soils exposed to a repeated pattern of soil drying and rewetting may be more important than the increase in leaching during soil rewetting events. My results suggest that the highest losses of nitrogen will occur when soils dry down enough to limit hydraulic connectivity, but not enough to severely limit microbial nitrification, creating pools of mobile nitrate that readily leach out of soils during rewetting events.

## Introduction

Precipitation patterns are becoming more variable across the world (Karl et al. 1995, Easterling 2000) and this change is likely to affect ecosystem processes including nutrient retention (Knapp et al. 2008). Increases in precipitation variability (less frequent, more intense precipitation) can increase the occurrences of soil moisture extremes, and wet-dry cycles. Wet and dry soil moisture cycles affect important components of nutrient retention relative to consistently moist soils — microbial activity (Davidson et al. 1993, Shen et al. 2008), the mobility of nutrients in soils (Lambers et al. 2008) and plant uptake (Cui and Caldwell 1997) — and have been correlated to decreases in nitrogen retention across a range of Long-Term Ecological Research sites (Kane et al. 2008).

Plant uptake is one of the most important components of nutrient retention and changes in plant uptake could affect nutrient retention at large scales. Most plants rely on mycorrhizal symbioses to extract and retain nutrients and sometimes water from their surroundings (Auge 2004). Arbuscular mycorrhizae (AM), the most common form of this symbiosis are known to extract phosphorus from soils and have been shown to reduce leaching losses of phosphorus in experimental mesocosms (van der Heijden 2010). Ectomycorrhizal fungi (EM) associate with far fewer species of plants, but occupy a disproportionately large area of terrestrial ecosystems (Smith and Read 2008). Ectomycorrhizae have the ability to decompose organic matter and directly extract nitrogen (Talbot et al. 2008), and typically thrive where inorganic nitrogen availability is limited (Read and Perez-Moreno 2003). The differences in how AM and EM mycorrhizae interact with nitrogen appears to feed back through the nitrogen cycle by reinforcing a particular nitrogen economy (Phillips et al. 2013). Arbuscular mycorrhizae reinforce a nitrogen cycle based predominately on inorganic nitrogen, whereas ectomycorrhizae reinforce an organic nitrogen based cycle. Inorganic nitrogen, especially nitrate, is typically more mobile in the soil than organic nitrogen, so an arbuscular mycorrhizal nitrogen economy would be expected to suffer higher leaching losses than the organic nitrogen economy created by ectomycorrhizae. We know that precipitation variability impacts nitrogen retention at large scales (Kane et al. 2008), that arbuscular mycorrhizae can limit the losses of phosphorus from soils (van der Heijden 2010) and that ectomycorrhizal dominated systems typically have less mobile forms of nitrogen in the

soil (Phillips et al. 2013). However, the role ectomycorrhizae in limiting the loss of nitrogen from ecosystems experiencing more extreme precipitation patterns remains unknown.

The purpose of this study was to test the effects of precipitation variability and mycorrhizal associations on nitrogen retention. I hypothesized that low-frequency but high-intensity rainfall would result in higher fluxes of nitrogen in leachate, especially  $\text{NO}_3$ . There are two ways that nitrogen retention might respond to precipitation variability, first through the response to individual events and second through the response to a long-term pattern of events. I hypothesized that nitrogen fluxes in leachate would respond similarly to both events and long-term patterns of rainfall. In other words, I expected larger leachate fluxes from drier soils following rewetting, than from consistently moist soils, and that soils with more soil moisture variability — repeated drying and rewetting — would release more nitrogen than consistently moist soils. The low-frequency treatment should increase nitrogen fluxes over the high-frequency treatment in response to rewetting events because wet and dry cycles should reduce the effectiveness of nitrogen retention mechanisms like plant uptake, and microbial immobilization, and the repeated pattern of dry and wet cycles should consistently minimize nitrogen retention resulting in higher fluxes in soils experiencing more soil moisture variability. I hypothesized that ectomycorrhizae would reduce nitrogen leaching relative to arbuscular mycorrhizae. Furthermore, I expected to see less of an effect of rainfall treatment in the ectomycorrhizal than the arbuscular mycorrhizal treatments because ectomycorrhizae can decompose and uptake soil nitrogen and reinforce an organic nitrogen based nitrogen cycle, potentially reducing the amount of substrate susceptible to leaching.

I measured the effects of rainfall variability and mycorrhizal type using small plant mesocosms in a greenhouse experiment. I measured nutrients in soil leachate from cottonwood (*Populus deltoides*) seedlings inoculated with either arbuscular or ectomycorrhizae and grown under low- or high-variability watering regimes. I used cottonwood seedlings for their somewhat unusual ability to associate with either arbuscular or ectomycorrhizal fungi, allowing me to hold species as a constant in this experiment. The rainfall treatments had a variety of effects on soil moisture, so I used regressions against continuous soil moisture measurements and statistics to interpret the effects of the rainfall treatment.

## Methods

### Plant growth and treatments

I used small plant mesocosms to measure the effects of rainfall variability and mycorrhizal type. Most plants associate with either arbuscular or ectomycorrhizal fungi (Smith and Read 2008). So to ease comparison of mycorrhizal treatments I chose Eastern Cottonwood (*Populus deltoides*) as a model organism for its ability to associate with either arbuscular or ectomycorrhizal fungi. I collected 72 cuttings of Eastern Cottonwood from a single tree near Northfield, MN (44.408681° N 93.041098° W) on 27 July, 2011, of which 21 survived through overwintering until the beginning of the rainfall treatments. To increase replication I collected an additional 51 cuttings from the same tree on 7 May, 2012, of which 19 survived to the beginning of the rainfall treatments. I rooted all cuttings by dipping them in rooting hormone (Hormodin 1) and placing them in wet sand. After rooting, I transplanted cuttings to 10 cm diameter tree pots in a 6:3:2 mix by volume of sterilized agro-mix (composted manure, top soil, peat), vermiculite and sand. Cuttings from 2011 were overwintered for 50 days below 6 °C. I inoculated the cuttings with either ecto- (EM, n = 21) or arbuscular-mycorrhizal (AM, n = 16) fungi (DIEHARD Endo Drench or Ecto Drench, Horticultural Alliance, Sarasota, FL). The ectomycorrhizal inoculum contained *Pisolithus tinctorius* and *Rhizopogon*, and the arbuscular mycorrhizal inoculum contained *Glomus mosseae*, *Glomus intraradices*, *Glomus fasciculatum*, *Glomus dussii*, *Glomus clarum*, *Glomus deserticola*, and *Glomus microaggregatum*.

I began experimental water treatments on 21 June, 2012. Half of the plants in each group received frequent, smaller waterings of 100 to 400 mL (1 to 4 mm), three times a week. The remaining half received less regular, larger waterings, typically 300 to 400 mL (3 to 4 mm), once to twice a week. Watering amounts varied slightly by date within a treatment to ensure the same total water in the high and low frequency treatments at each of the leachate measurement dates. All treatments received the same overall quantity of water at each leachate measurement date and at the end of the experiment (6300 mL or 63 mm, Table 3.1). I continued the water treatments until 17 September, 2012 (for a total duration of 88 days). I did not adjust watering volumes to compensate for differences in evapotranspiration between individual pots, instead I measured soil

moisture (Campbell Scientific Hydrosense, 12 cm probe) before each watering and used that as a predictor variable in the regressions (Figure 3.1).

At approximately 4 week intervals I watered all plants with 400 mL and collected leachate filtered through glass wool from the bottom of each pot. After 30 minutes of leachate collection I mixed the leachate well and saved 20 mL. Some pots occasionally required more than 400 mL of water to produce enough leachate. To account for the differences in watering volumes I multiplied the volume of water used by the concentration of solute in the leachate and reported the result as a flux ( $\mu\text{g}$ ). This approach did not allow for the detection of effects based on differential flow paths, possible dilution effects or differences in percolation rates between pots. I filtered all leachate samples through  $5\mu\text{m}$  pre-filters and  $0.7\mu\text{m}$  GF/F filters and stored them frozen before chemical analysis. I also measured longest leaf length (mm), stem diameter (mm), and height (cm) on each leachate collection date (Figure 3.2). After the final watering I harvested plants for biomass and collected a soil sample. Visual inspection of roots at harvest confirmed the presence of short, thick root tips indicative of ectomycorrhizal infection, in only the ectomycorrhizal treatment.

## Analytical methods

I measured soil moisture with a Campbell Scientific Hydrosense (12 cm probe) before each watering. I analyzed all filtered leachate samples for total organic carbon (TOC), total dissolved nitrogen (TDN) (Shimadzu TOC V), nitrate + nitrite ( $\text{NO}_3 + \text{NO}_2$  —  $\text{NO}_3$  from now on) using a spectrophotometric determination with vanadium reduction and sulfanilamide reagent (Doane and Horwath 2003) and  $\text{NH}_4$  using spectrophotometric determination with phenol-hypochlorite (Weatherburn 1967). I calculated dissolved organic nitrogen (DON) as  $(\text{TDN} - \text{NO}_3 - \text{NH}_4)$ . I counted and weighed leaves, stems and roots oven dried at  $65^\circ\text{C}$ . I measured plant height, stem diameter, longest leaf length, and number of leaves at each leachate collection date to estimate biomass based on a statistical relationship between those measurements and final harvested biomass as described below. After the final leachate collection I harvested the plants and measured biomass from harvested leaves, stems and roots.

## Biomass and growth rate estimates

To estimate above and belowground biomass at each leachate collection date I used measurements of plant height, stem diameter, leaf number and longest leaf length to build a statistical relationship with the final harvested biomass. I developed separate models to estimate above and belowground biomass for the dates that I measured stem diameter, leaf length, leaf number, longest leaf and stem length. I excluded stem diameter measurements from the models due to high measurement errors. After choosing model weights and random effects structures to meet model validation criteria, I used backward model selection by AIC to select the best fit model. The final model for aboveground biomass was:  $Aboveground\ Biomass = 2.44 + 0.03*Height + 1.67*Stem\ Diameter + 1.61*Number\ of\ Stems - 0.59*Stem\ Diameter:Number\ of\ Stems$ . The best model for belowground biomass was:  $Belowground\ Biomass = 5.45 - 4.39*AgeTrt + 0.004*Longest\ Leaf - 0.13*Number\ of\ Leaves + 0.003*Longest\ Leaf:Number\ of\ Leaves$  with a random effect on the intercept of rainfall treatment and weights defined by the variances within age groups. For each plant measurement date I calculated the daily mean relative growth rate ( $mg\ g^{-1}d^{-1}$ , Hunt 1982 p. 18) of above and belowground biomass.

## Statistical methods

I followed the mixed effects modeling approach outlined by Zuur et al. (2009) to account for differences in variances across treatments, correlations through time and random effects where applicable. I used soil moisture, cumulative soil moisture variance (the variance of soil moisture in a pot from all previous dates until each of the leachate collection dates) and cumulative mean soil moisture (the average soil moisture in a pot from all previous dates until each of the leachate collection dates) as continuous predictors in most of the statistical models to account for the different ways in which the rainfall treatment affected soil moisture. Differences in plant biomass, plant location on the benches, or other factors may have resulted in different evapotranspiration rates from pots, so I used the previously reported soil moisture statistics as a measure of treatment effect rather than the high- or low-frequency treatment designation. I analyzed dissolved nitrogen and organic carbon fluxes across all leachate collection

dates. Because I collected some plants in 2011 and other in 2012, I used plant age as a random effect in a linear mixed-effects model. Repeated measurements on the same subjects (four leachate collection dates) introduced the possibility of correlation between measurements, so I also tested a series of correlation structures to avoid violating the independence assumption. I used Akaike information criterion (AIC) scores to compare different random effects formulations, weighting structures to reduce heteroscedasticity (differences in variances of nutrient fluxes by treatments) and correlation structures (repeated measures). Fixed effects were selected using backwards model selection by AIC. I used the same statistical methods for all flux and biomass models including models of aboveground biomass, root biomass and growth rates. I added biomass covariates to the final flux model to test for the effects of aboveground, root or total biomass on the fluxes. For all mixed effects models I report  $R^2$  as the  $R^2$  of a linear model of the fitted mixed-effects model values against the measured values. A general approach for calculating  $R^2$  for mixed effects models exists, but does not currently work for models with weights (Nakagawa, personal communication, 25 June, 2013, Nakagawa and Schielzeth 2012).

The initial fixed effects for the flux model were the full factorial combination of *solute*, *rainfall treatment*, *mycorrhizal treatment*, *VWC*, *cumulative soil moisture variance* and *cumulative mean soil moisture*. Backwards model selection removed the six-way interaction, all five-way interactions involving solute, all four-way interactions involving solute except *Solute:RainTrt:VWC.var:VWC.mean* and one three-way interaction, *Solute:VWC:VWC.var*, and reduced the AIC score from 12516.79 to 12436.37. The flux model used weights based on solute and a random intercept and slope for soil moisture variance grouped by plant age nested in solute. The final flux model fit the data with  $R^2 = 0.87$ .

All data analysis and statistics were done with R version 3.0.1 (2013-05-16) (R Core Team 2013). I fit models using *gls* and *lme* from the `nlme` package (Pinheiro et al. 2013) and *gam* from `mgcv` (Wood 2011). I performed model selection using *stepAIC* from the `MASS` package (Venables and Ripley 2002).



## Results

### Overview

In response to individual rewetting events, drier soils from low-frequency watering released more nitrate and total nitrogen than wetter soils. Ectomycorrhizal treatments slightly reduced the effect of antecedent soil moisture on total nitrogen and nitrate losses from rewetting events. This supports my first hypothesis that drier soils release more nitrogen after rainfall events. However, only  $\text{NH}_4$  increased in proportion to the cumulative variance in rainfall quantity and mycorrhizal treatments had no effect, largely refuting my hypothesis that soils would release more nitrogen when exposed to higher variability patterns of rainfall. These results did not depend on differences in aboveground, root or total plant biomass. Antecedent soil moisture and cumulative mean soil moisture were the most important factors influencing different solute fluxes.

The rainfall treatments resulted in 50% higher cumulative soil moisture variance in low frequency treatments ( $F_{1,35} = 6.94$ ,  $p = 0.01$ , Figure 3.3). As intended, the rainfall treatments had no effect on cumulative mean soil moisture ( $F_{1,35} = 0.14$ ,  $p = 0.71$ , Figure 3.3). Fluxes were composed of 43% nitrate, 2% ammonium and 54% organic nitrogen averaged across all leachate collection dates and treatments.

### Rainfall, mycorrhizae and fluxes

To determine the response of soils to one-time rewetting events I compared the relationship between nitrogen fluxes on rewetting and soil moisture immediately prior to rewetting. Dissolved organic carbon,  $\text{NH}_4$ , and DON all increased in proportion to antecedent soil moisture, while TN and  $\text{NO}_3$  decreased in proportion to antecedent soil moisture (Figure 3.4). There was no effect of mycorrhizal treatment alone on solute fluxes ( $F_{4,615} = 1.68$ ,  $p = 0.15$ ). However, ectomycorrhizal treatments interacted with antecedent soil moisture to slightly reduce the effect of antecedent soil moisture on nitrate and TN fluxes ( $F_{4,615} = 3.78$ ,  $p = 0.005$ , Figure 3.4). The decreases in TN and  $\text{NO}_3$  fluxes at higher antecedent soil moisture confirms my hypothesis that drier soils release more nitrogen, and especially  $\text{NO}_3$  upon rewetting. Furthermore, ectomycorrhizae appear to reduce the effects of rewetting events on total nitrogen and nitrate, as hypothesized.

I used cumulative soil moisture variance, the variance of soil moisture measurements up to a date of interest, to measure of the effects of rainfall pattern on nitrogen fluxes. Ammonium fluxes increased in proportion to the cumulative variance in soil moisture, while DON, TN and  $\text{NO}_3$  decreased in proportion to cumulative soil moisture variance ( $F_{4,615} = 2.51$ ,  $p = 0.04$ , Figure 3.5). Mycorrhizal treatment had no effect, except on dissolved organic carbon, which increased slightly in ectomycorrhizal treatments but did not change in proportion to cumulative soil moisture variance in arbuscular mycorrhizal treatments ( $t_{615} = 2.58$ ,  $p = 0.01$ , Figure 3.5). Contrary to my hypotheses, total nitrogen and nitrate *decreased* in response to more variable rainfall patterns, suggesting that reoccurring pattern of drying and rewetting does not consistently minimize the effectiveness of nitrogen retention mechanisms. The results from the mycorrhizal treatments also refuted my hypothesis that ectomycorrhizal associations reduced nitrogen losses caused by high variability rainfall patterns.

I also used cumulative mean soil moisture, or the average of soil moisture measurements up to a date of interest, to measure the effects of rainfall pattern. Fluxes of TN and  $\text{NO}_3$  increased in proportion average soil moisture, while DOC and  $\text{NH}_4$  decreased ( $F_{4,614} = 21.21$ ,  $p < 0.001$ , Figure 3.6). There was an interactive effect of mycorrhizal treatment and cumulative mean soil moisture on fluxes. Fluxes of DON,  $\text{NO}_3$  and TN increased with increasing cumulative mean soil moisture (*% by volume*) in ectomycorrhizal treatments but remained constant in arbuscular mycorrhizal treatments ( $F_{4,615} = 3.55$ ,  $p = 0.007$ ). In contrast, fluxes of  $\text{NH}_4$  remained constant through changes in cumulative mean soil moisture and across mycorrhizal treatments (Figure 3.6).

## Biomass and fluxes

Larger plants could have a stronger effect on soil fluxes through either root processes like uptake and turnover, or higher evapotranspiration. I added biomass covariates to the final flux model to test for effects of differences in plant biomass. The final flux model was not improved by the addition of aboveground ( $\Delta\text{AIC} = 0.89$ ,  $p = 0.29$ ), root ( $\Delta\text{AIC} = 1.03$ ,  $p = 0.14$ ) or total biomass ( $\Delta\text{AIC} = 2.12$ ,  $p = 0.92$ ) and the addition of biomass changed neither the strength nor direction of any of the relationships between fluxes and events or patterns of soil moisture (Figure 3.7). The lack of influence of plant biomass suggests that the results of the rainfall and mycorrhizal treatments reflect

differences in those treatments more than individual variation between plants in biomass and evapotranspiration.

## Discussion

My results suggest that rewetting events but not repeated patterns of events were important drivers of total nitrogen and nitrate fluxes and that ectomycorrhizae have very limited potential to reduce nitrogen losses caused by rewetting events or patterns of drying and rewetting. Total nitrogen and nitrate fluxes increased in proportion to soil dryness in response to rewetting events, but decreased in response to increased soil moisture variability. Ectomycorrhizal associations were mildly effective at reducing total nitrogen and nitrate fluxes in response to rewetting events, but had no effect on nitrogen fluxes in response to patterns of rewetting.

### Rainfall events, patterns and fluxes

The increased nitrate and total nitrogen fluxes in response to rewetting events in drier soils are consistent with both small-scale studies on soil drying and rewetting cycles and large-scale studies on precipitation and stream fluxes. Soil drying and rewetting events often temporarily increases the availability of nitrate in the soil (Davidson et al. 1993). The fate of that new nitrate has been uncertain but upon rewetting could include plant and microbial uptake, denitrification and soil leaching. Higher nitrate and total nitrogen fluxes at lower antecedent soil moisture are consistent with microbial mineralization during soil dry-down (Singh et al. 1989) and subsequent rapid nitrification following rainfall events (Davidson et al. 1993).

The pattern of repeated drying and rewetting events decreased total nitrogen and nitrate leaching, and seems to contradict the role of drying and rewetting cycles in driving nitrogen fluxes in response to events. However, higher soil moisture variance suggests longer periods of dry soil than in soils with lower soil moisture variance but the same total quantity of rainfall. Lower soil moisture reduces microbial nitrification activity by reducing the availability of substrates through diffusion limitation and reducing cell hydration (Stark and Firestone 1995). Longer periods of drought could therefore reduce overall nitrification compared to consistently moist soils. This reduction of nitrification

has been shown previously in soils associated with cowpeas exposed to repeated soil rewetting (Franzluebbers et al. 1994, but see Fierer and Schimel 2002). Similarly, the pattern of multiple rewetting cycles has decreased nitrate and increased ammonium relative to consistently moist grassland soils (Xiang et al. 2008) and suggests a limitation of nitrification in the soils exposed to a pattern of drying and rewetting. The increase in ammonium observed by Xiang and others (2008) is similar to the increase in ammonium fluxes I observed under increased soil moisture variance. This increase in ammonium flux may be a sign that the soils in this experiment that experienced a pattern of drying and rewetting had reduced nitrification. An overall reduction in nitrification would resolve the apparent contradiction between increased nitrate fluxes caused by rewetting events and decreased nitrate fluxes caused by patterns of drying and rewetting. These results further suggest that when exposed to a repeated pattern of soil drying and rewetting, the degree and duration of drying experienced by soils may be more important than the increase in leaching caused by soil rewetting events.

My results suggest that the highest losses of nitrogen will occur when soils dry down enough to limit hydraulic connectivity, but not enough to severely limit microbial nitrification. Because of this sensitivity to the degree of drying, baseline climate may play an important role in understanding the effects of increased rainfall variability (Knapp et al. 2008). Moist climates might be expected to experience larger nitrogen losses under more variable precipitation than either very-wet or very-dry climates. In very-wet climates soils might not dry down enough to impact plant and microbial activity, whereas in xeric climates dry soils would be the norm and additional variability would not further decrease plant and microbial activity between rewetting events. The best evidence to support or refute this idea comes from a synthesis of precipitation variability and inorganic nitrogen retention from LTER sites across the United States (Kane et al. 2008). Across these sites higher precipitation variability (expressed as coefficient of variation) resulted in lower inorganic nitrogen retention at the watershed scale. However, Kane and others (2008) found no effect of mean annual precipitation on the relationship between precipitation variability and inorganic nitrogen retention across all seasons. While there was no effect across all seasons, increased mean annual precipitation (between about 200 and 2400 mm) did increase nitrogen retention during the summer growing season. The summer growing season would be expected to have the highest plant and microbial

activity, and therefore the largest effect of mean annual precipitation and precipitation variability, lending support to the results of this experiment.

At least two potentially overlapping mechanisms have been proposed to explain large fluxes of nitrogen during floods or other intense rainfall events: (1) soil accumulation of soluble nitrogen compounds during dry periods that are subsequently flushed during rain events (Meixner et al. 2007), and (2) flushing of nitrogen compounds from newly wetted flow paths (Creed and Band 1998). In this study the source area remains constant (pot volume) between nitrogen flux measurements, so the changes in fluxes should result from changes in accumulation between flushing events. Pots that remained wetter between rewetting events may have leached more nitrate in the long run due to higher microbial nitrification in consistently moist soils (Xiang et al. 2008).

### **Mycorrhizae and fluxes**

Contrary to expectations, ectomycorrhizal fungi alone or in combination with soil moisture variance or antecedent soil moisture did not affect nitrogen fluxes. However, ectomycorrhizal treatments did increase fluxes of DON, NO<sub>3</sub> and TN in proportion to cumulative mean soil moisture. Droughts have been shown to reduce ectomycorrhizal colonization in Norway Spruce (Nilsen et al. 1998) and fungal decomposition (Manzoni et al. 2012). Consistently higher soil moisture should increase ectomycorrhizal activity, potentially increasing the availability of nitrogen compounds for leaching, but also the assimilation and retention by mycorrhizae (Stark 1972). A possible explanation for the increase in DON, NO<sub>3</sub> and TN fluxes is that occasional periods of drought, even in generally wetter soils may have increased the turnover of ectomycorrhizal hyphae which had accumulated nitrogen compounds, thus returning those compounds back to the soil pool available for leaching.

As expected, arbuscular mycorrhizal fungi had no effect on nitrogen fluxes. In general, arbuscular mycorrhizae do not decompose soil organic matter or assimilate nitrogen (but see Hodge et al. 2001, Tu et al. 2006), and tend to exist in soils with an inorganic nitrogen economy (Read and Perez-Moreno 2003, Phillips et al. 2013). These results are consistent with previous leaching experiments demonstrating that arbuscular mycorrhizae can reduce leaching of phosphorus, but have no effect on nitrogen losses (van der Heijden 2010).

## Conclusion

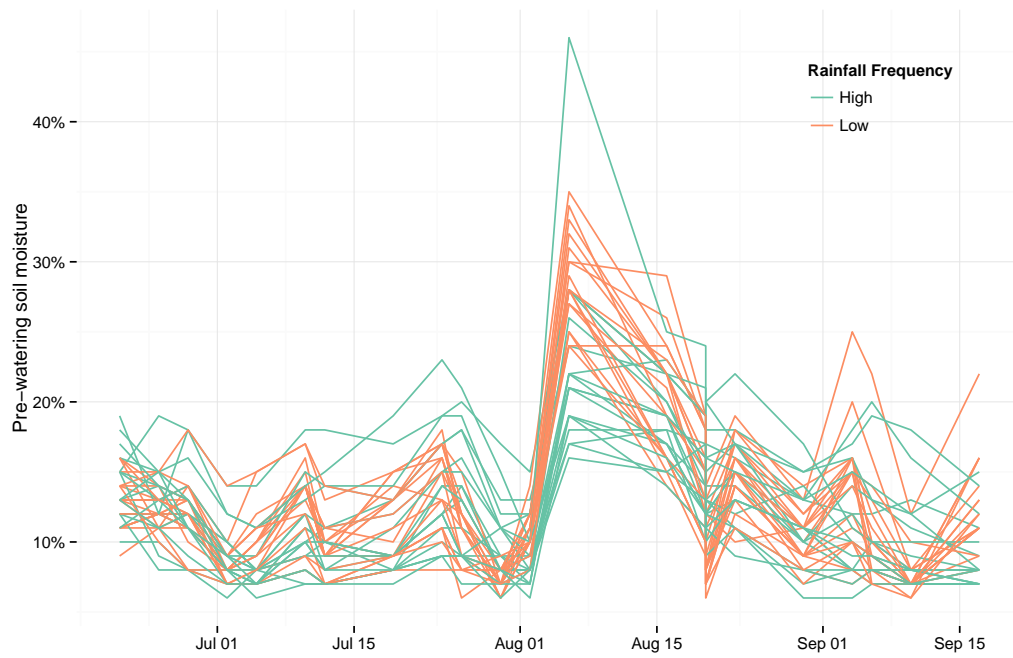
Rewetting events but not patterns of repeated events were important drivers of total nitrogen and nitrate fluxes in this experiment. Total nitrogen and nitrate fluxes increased in proportion to soil dryness in response to rewetting events, but decreased in response to increased soil moisture variability. The degree and duration of drying in soils exposed to a repeated pattern of soil drying and rewetting may be more important than the increase in leaching during soil rewetting events. Additionally, ectomycorrhizal associations were mildly effective at reducing total nitrogen and nitrate fluxes in response to rewetting events, but had no effect on nitrogen fluxes in response to patterns of rewetting. My results suggest that the highest losses of nitrogen will occur when soils dry down enough to limit hydraulic connectivity, but not enough to severely limit microbial nitrification, creating pools of mobile nitrate that readily leach out of soils during rewetting events.

## Tables

**Table 3.1** Averages and standard deviations of watering volume and days between waterings for high (H) and low (L) rainfall treatments

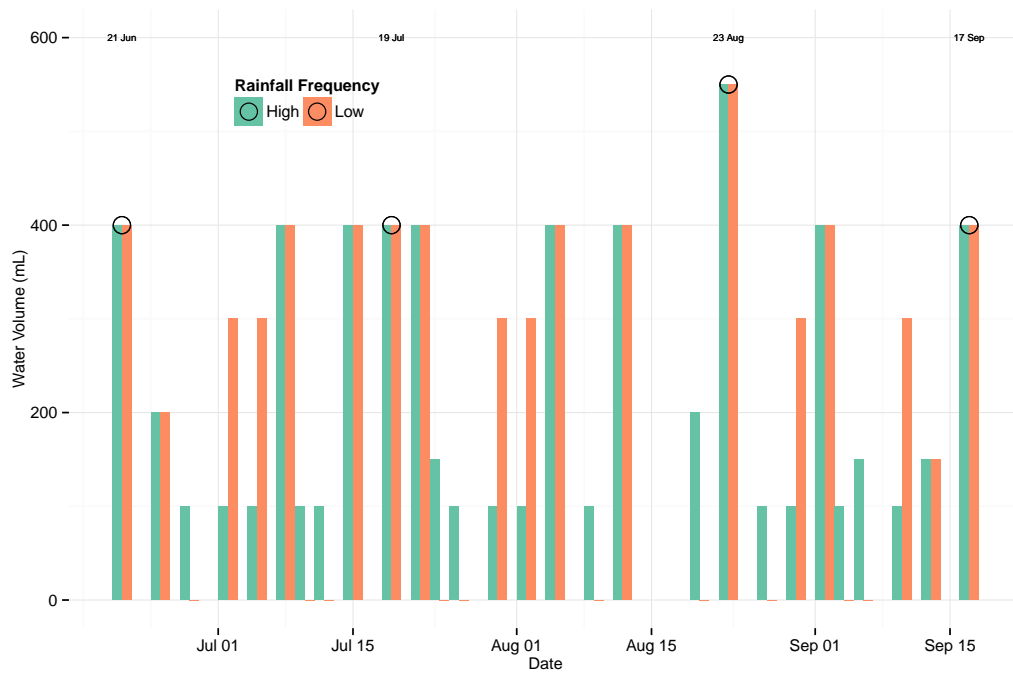
RainTrt	Avg. Volume	SD Volume	Avg. Days	SD Days	Total Volume
H	225.00	149.38	3.26	1.20	6300
L	350.00	90.75	5.18	2.51	6300

## Figures

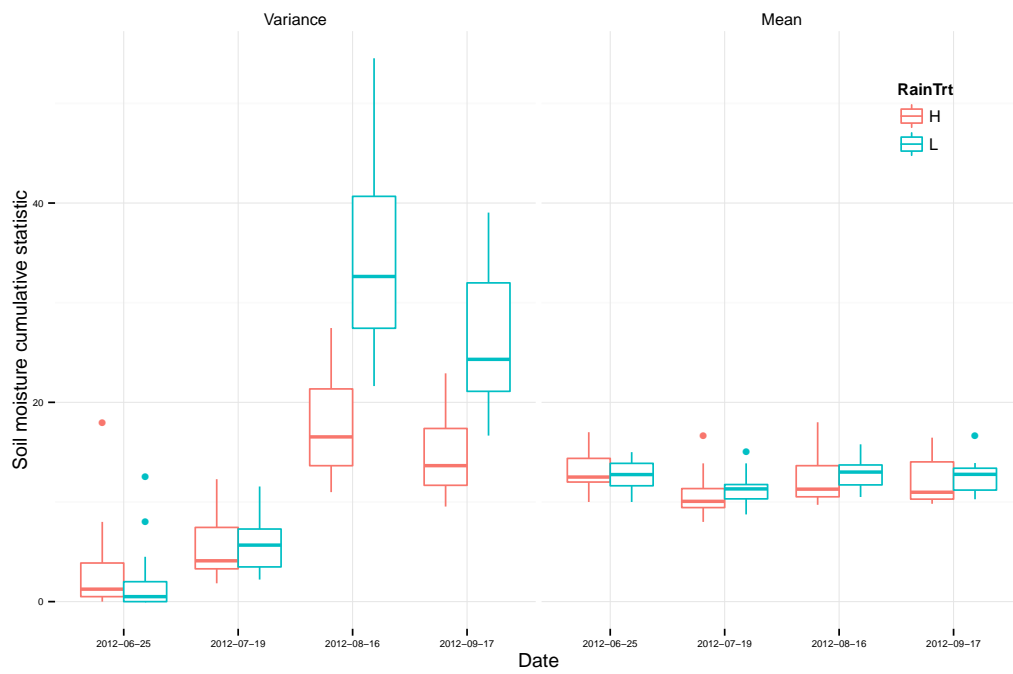


**Figure 3.1** Pre-watering soil moisture (*% by volume*)

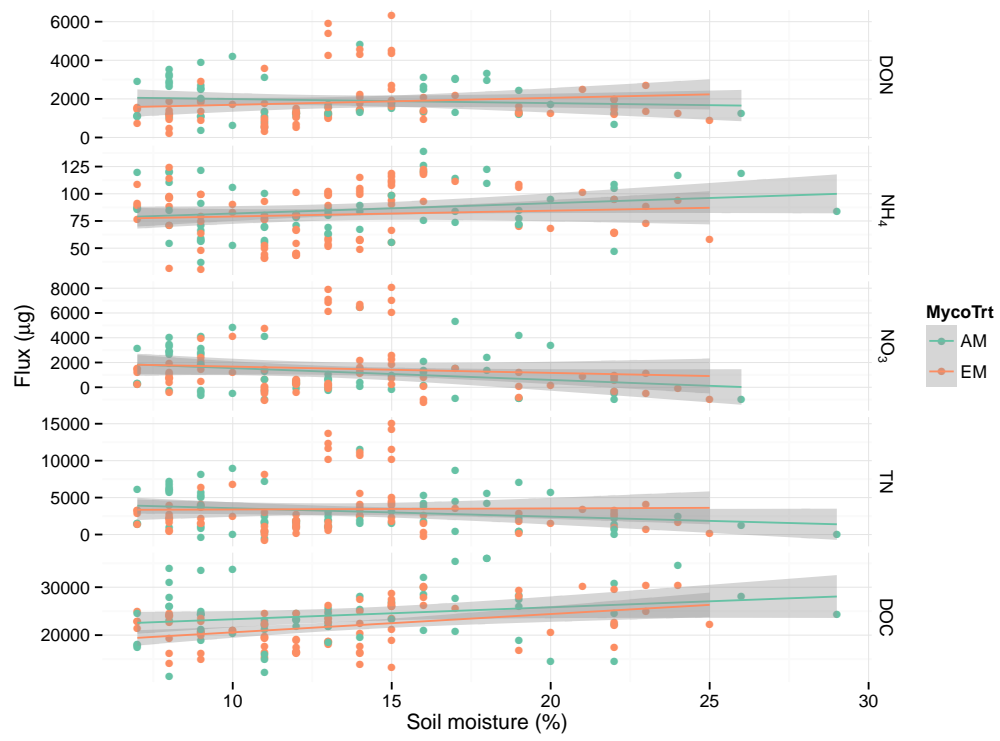




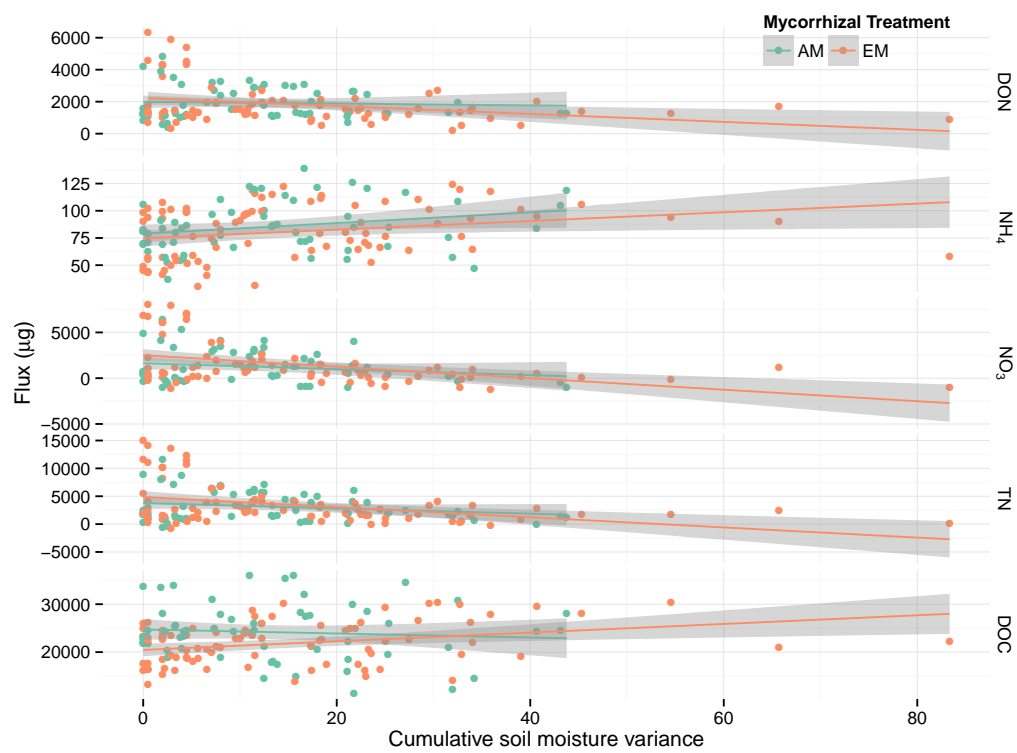
**Figure 3.2** Watering schedule, circles indicate dates of throughflow measurement



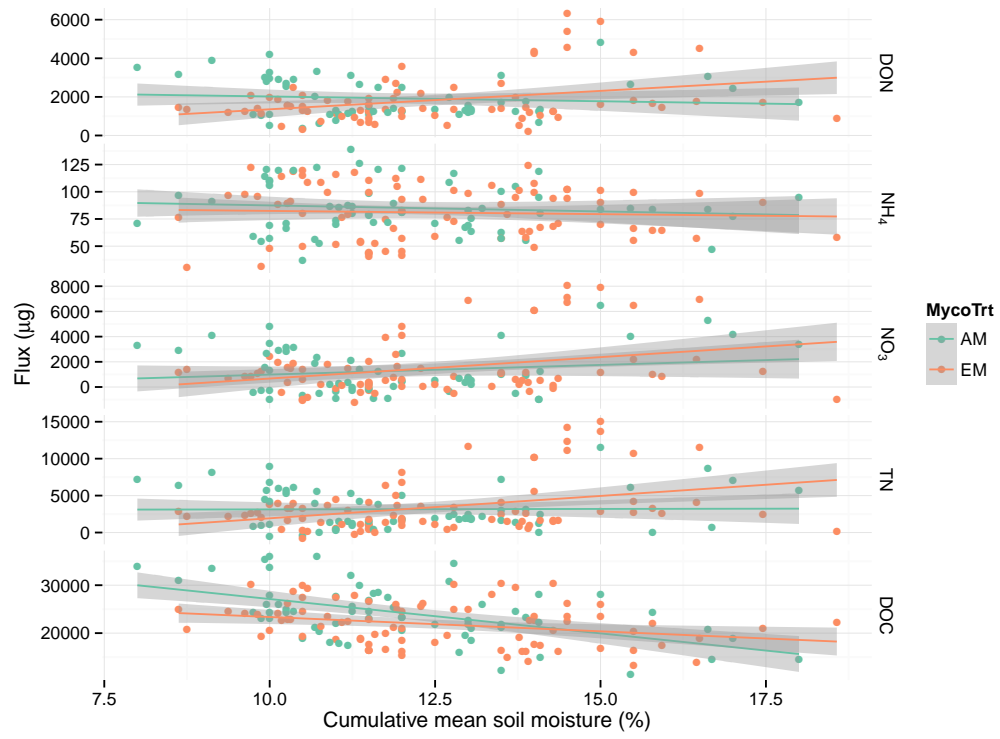
**Figure 3.3** Cumulative variance and mean soil moisture by rainfall treatment (H: high frequency, L: low frequency)



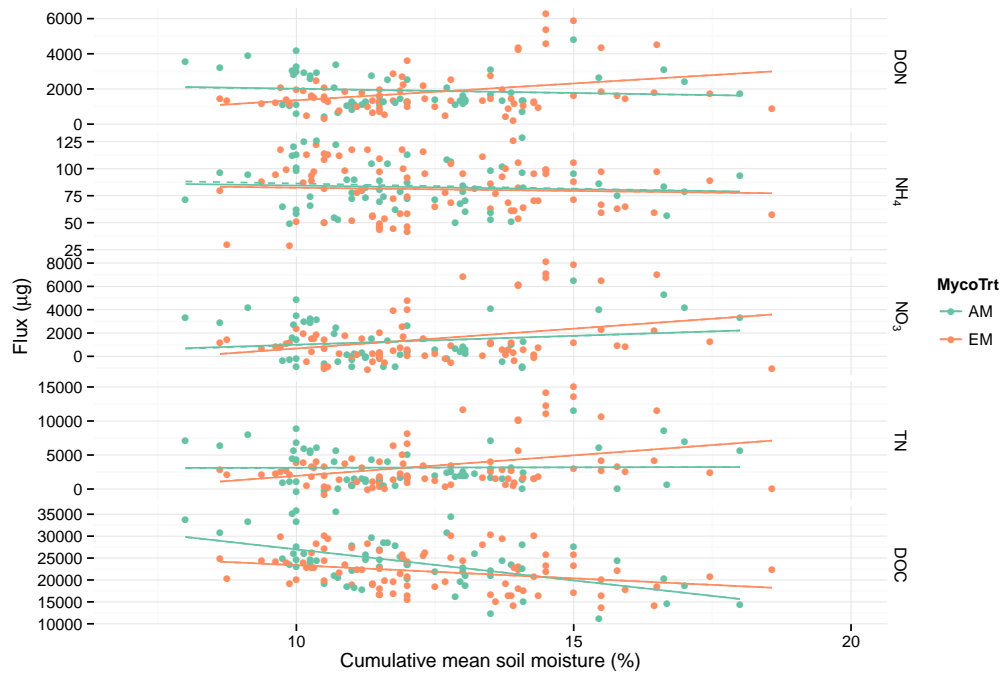
**Figure 3.4** Fluxes ( $\mu\text{g}$ ) as a function of antecedent soil moisture (% by volume)



**Figure 3.5** Fluxes ( $\mu\text{g}$ ) as a function of cumulative soil moisture variance



**Figure 3.6** Fluxes ( $\mu\text{g}$ ) as a function of cumulative mean soil moisture (*% by volume*)



**Figure 3.7** Fluxes ( $\mu\text{g}$ ) with root biomass covariate ( $g$ ). Solid lines indicate model fit with root biomass covariate, dashed lines indicate model fit without.

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## Chapter 4

# Making it fit: an assessment of strategies to mitigate the water quality impacts of nitrogen from meeting the needs for food, fuel and other goods to consume in 2050

Joseph Pignatello Reid<sup>1\*</sup>, Kate A. Brauman<sup>2</sup>, Stephen Polasky<sup>1,3</sup>

<sup>1</sup> Department of Ecology, Evolution & Behavior, University of Minnesota - Twin Cities

<sup>2</sup> Institute on the Environment, University of Minnesota - Twin Cities

<sup>3</sup> Department of Applied Economics, University of Minnesota - Twin Cities

\* jreid@umn.edu

Keywords: global sustainability, nitrogen, phosphorus, levers

*JPR designed and implemented the model, designed the experiment, carried out the research and wrote the manuscript, KAB provided guidance in model design and helped*

*interpret results, SP conceived of the project*

## Abstract

Growing populations and increasing wealth are expected to double food demand and increase the demand for energy and goods and services. Meeting the needs for food, energy and goods and services for future generations is likely to come with enormous environmental costs. The widespread degradation of water quality through nitrogen pollution is one such environmental cost that currently impacts human well-being. We ask three key questions about the future of water quality related to changes in nitrogen management: 1) how might the future compare to the present? 2) where are the places that are most sensitive to changes in technologies, mitigation strategies, and population and economic growth? 3) Can these technologies and strategies help us meet future demands while maintaining or even improving current water quality? To answer these questions we developed an integrated model of the world's energy, food and economic demands tightly coupled to environmental quality. Unlike other integrated assessment models we developed ours explicitly for the purposes of testing technological and strategic levers for the world in 2050. The levers tested were focused on agriculture — genetic gains to crop yields, nutrient use efficiency (precision agriculture), meat consumption, food waste — and wastewater treatment, the shift toward higher treatment levels and nitrogen removal in wastewater treatment plants.

The levers we tested were marginally effective at reducing nitrogen yield (kg of nitrogen exported in rivers per km<sup>2</sup> per year) from the baseline business as usual (BAU) scenario, typically less than 2%. Reducing food waste by half (from 30% to 15%) reduced demand for food crops and meat, and reduced cropland area, resulting in 2.2% reductions in nitrogen yield from BAU. The other levers applied singly were less effective at reducing nitrogen yield. Not all levers were effective everywhere: the largest impacts were in Asia (by reducing cropland expansion, increasing nutrient application efficiency and improving wastewater treatment) and Africa (by reducing cropland expansion and improving wastewater treatment). The smallest effects were in Oceania which had the lowest proportion of total nitrogen yield from agricultural sources. Most agricultural levers were effective in Northern America through reductions in fertilizer applications and cropland expansion. Applied in combination, in the “Super Ag” scenario, the levers outperformed the sum of their contributions when applied singly by 14%. Although the

“Super Ag” scenario decreased global nitrogen yield from BAU by 6.5%, there was still a 15% increase in global nitrogen yield from the present. As illustrated by the combined agriculture scenario, synergistic effects existed among levers. Efforts to address nitrogen pollution should increase the options available (levers) at the local level for reducing nitrogen demand and increasing the efficiency of nitrogen use. Generally, any lever that keeps land out of agriculture has potential to reduce the water quality impacts of providing for human well-being. Reducing the demand for marginal cropland by reducing calorie demand or increasing yields removes agriculture from lower yielding, higher fertilizer marginal croplands. The effect of reducing cropland area has the largest effect on reducing nitrogen yields in our scenarios and that effect is strongest in Africa and Asia where the largest expansion of croplands occurs.

## Introduction

Sustainable development requires providing for human well-being by meeting basic demands for food, energy and consumer goods and services, all while maintaining an environment capable of sustaining the provisioning of those demands for future generations (Brundtland Commission 1987, Holden and Linnerud 2007). Failure to meet the basic needs of human well being is not an ethically viable option and strategies for doubling agricultural production (Tilman et al. 2011, Foley et al. 2011) and providing energy (Edmonds and Reilly 1983) and goods for a growing population exist. However, the question is, at what cost to environmental quality?

Water quality is an important determinant of environmental quality. Nitrogen provides enormous benefits to society by increasing crop yields to feed the worlds population (Galloway et al. 2002). However, nitrogen pollution is a key contributor to degraded water quality that currently causes significant human health problems (Rabalais et al. 1996, Ward et al. 2005, Grosse et al. 2006), economic burdens and aesthetic problems (Camargo and Alonso 2006). Water quality is closely linked to the production of food, energy, goods and services required to provide a decent quality of life. For example, increasing agricultural production requires greater nutrient fertilization (Mueller et al. 2012), and fossil fuel combustion from growing energy demand in residential, industrial and transportation sectors results in atmospheric nitrogen pollution and deposition (Vitousek et al. 1997) leading to nitrogen export into water (Aber et al. 1998).

Current levels of nitrogen pollution already impact the quality of life for people. Nitrate contamination of drinking water can cause 'blue baby syndrome' in infants, and has recently been linked to a variety of cancers, even at levels below the EPA and WHO limits (10 mg/L and 11 mg/L nitrate-N respectively, Ward et al. 2005, Grosse et al. 2006, US EPA 2009, WHO 2011). Nutrient pollution also causes algal blooms which lead to massive coastal dead zones. Algal blooms can cause shellfish poisoning in coastal areas (Rabalais et al. 1996) and can contaminate freshwater supplies possibly increasing the risk of cancers (Grosse et al. 2006). The number of dead zones, or areas of the ocean with dissolved oxygen levels that are below the normal limits for marine animals, has nearly doubled every decade for the last 50 years (Diaz and Rosenberg 2008). Dead zones have been implicated in shifting habitat suitability away

from economically important fish species towards jellyfish, sometimes referred to as the “jellyfish joyride” (Richardson et al. 2009). In aquatic habitats nitrogen pollution can also lead to the production of  $N_2O$ , a potent greenhouse gas (Baron et al. 2013). Wastewater treatment can reduce the direct human contribution to water pollution, but costs scale in proportion to effectiveness of treatment and can represent a significant economic burden (Molinos-Senante et al. 2010).

Assessing current nutrient pollution is challenging. The problem is distributed globally and requires widespread water quality monitoring, which is limited in most of the world. Several models have been employed to assess current nutrient pollution and forecast future water quality impacts. Models such as GlobalNEWS2 (Bouwman et al. 2009, Seitzinger et al. 2010) used in the Millennium Ecosystem Assessment (MA), IMAGE (Bouwman et al. 2006), and the balance models of Liu and others (2010), and Green and others (Green et al. 2004) use nutrient budgets that track inputs and exports of nutrients to and from land. In these models agricultural inputs are scenario parameters that are not sensitive to potential technologies and strategies (levers) that could increase yields while reducing nutrient pollution (Foley et al. 2011, e.g. closing yield gaps, increasing nutrient use efficiency, Mueller et al. 2012). Additionally, both GlobalNEWS2 and IMAGE have been used to forecast changes in nutrient loading under various future scenarios. But these forecasts are based on FAO reports of what agriculture is likely to look like (Alexandratos et al. 2006), and cannot assess the potential utility of levers to reduce the environmental impacts of agriculture. There are a multitude of levers which could be used to reduce environmental harms across all components of these integrated assessment models, but the models have mostly been incapable of implementing them or they have not been used in this sense (for a limited implementation see Seitzinger et al. 2002).

We developed an integrated model of the world with feedbacks to water quality from demands for goods and services, energy, and food. In contrast to other integrated assessment models, our model was explicitly designed to test the potential for various levers to reduce the environmental harm produced as a consequence of providing for human well-being in 2050. We implemented levers to address proposed strategies for reducing nitrogen pollution including increasing nitrogen fertilizer use efficiency, decreasing nitrogen demand through changes in diet, reducing food waste and increasing access to

wastewater treatment (Galloway et al. 2008, Foley et al. 2011, Mueller et al. 2012). Our agriculture model is capable of implementing these key strategies to meet global food demands while minimizing environmental impacts (Mueller et al. 2012). This is in contrast to the static representation of the relationship between agriculture and the environment in IMAGE (Bouwman et al. 2006) and GlobalNEWS2 (Bouwman et al. 2009, Seitzinger et al. 2010). Similarly, we expand on the approach used by Green and others (Green et al. 2004) for predicting wastewater treatment effectiveness by explicitly incorporating a lever that accelerates the adoption of higher-level treatment systems.

Rather than ask what will be, we ask what is possible. As such we often assume optimal resources use, as in the case of agriculture, where crop demands are met in the most efficient manor without regard to land prices. We use the simplest possible methods for nutrient accounting to simplify model interpretation. Due to the darkness of the art of forecasting, we emphasize the primary purpose of this model is to compare scenarios and not to forecast the specific quantities of nutrient pollution in the future. As such we limit our reporting to large sub-continental regions.

We will attempt to answer three key questions about the future of water quality. 1) What could the future look like compared to the present? 2) How does water quality respond to the implementation of technologies and strategies to reduce nutrient inputs or decrease nutrient losses, and are there unavoidable impacts regardless of scenario parameterization? 3) To what extent can we improve water quality while providing the basic needs of a growing population?

## Methods

### The Make it Fit Model Framework

The Make it Fit model framework (*manuscript in preparation*) consists of modules for calorie demand, goods and services consumption, aquaculture and fisheries, agriculture food crops, water quantity, pasture raised food, energy, biofuels crops, timber, natural land cover and habitat, climate, air quality and water quality (Figure 4.1). The framework uses a five-minute resolution grid to track land use, agriculture, timber, water



quantity, and water and air quality. We briefly summarize the modules in Table 4.1.

## The Water Quality Model

We use a nutrient balance approach similar to Liu and other (2010), Green and others (2004), GlobalNEWS2 (Mayorga et al. 2010) and IMAGE (Bouwman et al. 2006) to track nitrogen flows and accumulations from atmospheric deposition, cropland agriculture, livestock, aquaculture, energy production and human populations. Diffuse inputs are partitioned into retained and runoff components using a nitrogen retention function. Municipal wastewater point sources receive some level of wastewater treatment, resulting in a retained (or eliminated) component and a runoff component. Finally, we combine and route downstream the point and non-point runoff components. We often use the simplest possible representation for any one source of nitrogen. This helps us avoid making highly uncertain assumptions about the distributions of inorganic and organic nitrogen by source, which we can not do for agriculture due to the limitations of that module. Using total reactive nitrogen also provides a more holistic representation of nitrogen in the environment (Galloway and Cowling 2002).

We consider fertilizer, atmospheric deposition, and biological nitrogen fixation as potential sources of diffuse nitrogen on the landscape. Manure and crop residues are derived from previously accounted for inputs (fertilizer, fixation or deposition) and are not tracked separately. We do not consider sediment from erosion in our nitrogen balance because it is <1% of the total nitrogen sources (Liu et al. 2010). We account for nitrogen exports in harvested crops (nutrient harvest ratios derived from Bouwman et al. 2011), leaching and retention for diffuse sources. We apply the model to all terrestrial land surfaces using our land-cover simulations from agricultural expansion, timber harvesting and urbanization. For non-agricultural land-uses we consider only biological nitrogen fixation and atmospheric deposition as diffuse sources, and leaching and retention as outputs. We account for direct stream nitrogen inputs from inland aquaculture and human wastewater. We simplify nitrogen balance and flux estimates by using total reactive nitrogen as our unit of accounting, rather than tracking dissolved inorganic and organic nitrogen separately.

Cropland fertilizer nitrogen was calculated by the agriculture intensification model to meet yield goals (Mueller et al. 2012). We assumed that 51% of the nitrogen fertilizer

applied to crops was harvested in the crops and that the remainder contributed to the nitrogen balance (Bouwman et al. 2011). For pastures we assumed that all nitrogen added to pastures was either retained in the pasture, leached out in surface water, or harvested in livestock. We removed the nitrogen content of livestock (assumed to be 15% protein by mass) from the fertilizers applied to improved pasture (Mueller et al. 2012) to calculate the pasture nitrogen balance.

Natural ecosystem biological nitrogen fixation rates for the major biomes of the world were estimated from the literature (Cleveland et al. 1999, Smil 1999). We mapped the estimated rates using a biome atlas (Olson et al. 2001) and masked out areas that were in agriculture, pasture or urban land uses. We took a similar approach for crop specific nitrogen fixation rates, using either published rates for individual crops (e.g., soy) or classes of crops (e.g., tubers; Smil 1999, Liu et al. 2010) and applying them to areas of each crop as reported by the agriculture model.

For nitrogen from aquaculture we used the nitrogen pollution rates estimated in the aquaculture model (Max Troell and Marc Metian, personal communication, 2013). We distributed aquaculture nitrogen based on water availability for each grid cell, with the highest rates going to the wettest grid cells in a country that are not already in agriculture.

Atmospheric  $\text{NO}_x$  and  $\text{NH}_y$  deposition rates for 1993 from Dentener (2006) were rescaled to match our five-minute grid. We scaled  $\text{NO}_x$  deposition rates by the change in  $\text{NO}_x$  emissions from 2005 to 2050 calculated by the energy model to each region. We held  $\text{NH}_y$  deposition constant to avoid double counting nitrogen deposition from fertilizers.

Human wastewater is the primary point source of nitrogen in our model. We estimated the nitrogen content of human waste by relating per capita GDP to protein consumption using FAO data (Figure 4.2, Van Drecht et al. 2009). We equate consumed nitrogen to excreted nitrogen by assuming steady state human biomass in 2050. Wastewater treatment removes a percentage of nitrogen and phosphorus defined by the mix of wastewater treatment facilities in a country, primary (mechanical), secondary (biological) and tertiary (advanced) (Van Drecht et al. 2009). The mix of wastewater treatment facilities is defined as a set of functions fit to the proportion of each treatment level in a country (EUROSTAT, WHO) by per capita GDP. The treatment

system proportions were used to calculate a weighted removal efficiency based on the removal efficiencies of each treatment level (Plappally and Lienhard 2012). Wastewater treatment infrastructure is assumed to improve with increasing per capita GDP, but never decreases if a country's per capita GDP decreases in a scenario.

We simulate nitrogen delivery in streams through the non-point sources of atmospheric deposition (driven in part by energy production), cropland fertilizer applications and biological nitrogen fixation, and livestock manure, as well as the point sources of human waste and aquaculture. Nitrogen is removed from the watershed nitrogen balance through crop harvesting, watershed nitrogen retention and wastewater treatment. Watershed retention coefficients are used to simulate the retention of nitrogen in soils, groundwater and riparian areas (Mayorga et al. 2010). The empirical watershed retention coefficients developed and calibrated by Mayorga and others (2010) consist of two constants to account for the positive correlation of nitrogen exports to both water runoff and nitrogen surplus and are calibrated to a global dataset of dissolved stream nitrogen fluxes. Through the global calibration with observed river nitrogen fluxes, the nitrogen retention coefficients implicitly account for the effects of soil texture, lakes, wetlands and other factors that influence nitrogen retention at a scale appropriate to our regional analysis. With these coefficients the fraction of nitrogen exported from a watershed increases in proportion to runoff, up to 1 m of runoff per year. One of the coefficients is a runoff exponent which describes the shape of the relationship between runoff and nitrogen export. The amount of the nitrogen surplus subject to export to streams is given by a second globally calibrated constant. We used the coefficients for dissolved inorganic nitrogen in general and agricultural areas resulting in a linear relationship between runoff and nitrogen export (runoff exponent = 1) and a maximum export of 94%. Runoff for the retention function is calculated as runoff without the effects of human interventions such as irrigation. We rescale a 30 minute global runoff map (Fekete et al. 2002) to our 5 minute grid for all runoff dependent calculations. Stream retention factors, also from Mayorga and others (2010) are applied to simulate in-stream removal. Results are reported in nitrogen yield ( $\text{kg N km}^{-2} \text{ year}^{-1}$ ) aggregated to continents. To calculate annual fluxes for validation purposes we route the exported nitrogen balance downstream using a flow direction map (Vörösmarty et al. 2000).

## Scenarios

We designed a number of scenarios to compare the effects of a variety of nutrient related levers (Table 4.2). We modified the following levers: population, gross domestic product (GDP), crop genetic gains, precision agriculture, diet, food waste and wastewater treatment. We used the United Nations population estimates for the low, medium and high fertility scenarios (United Nations 2013) and GDP estimates from PriceWaterhouse Coopers (2013) for 2050, except for the nowcast scenario which uses current 2008 GDP and population. Crop genetic gains improve the yields of crops per unit fertilizer and water by a proportion set in the scenario. The precision agriculture lever allows for reductions in fertilizer intensities in areas with application rates greater than required by the yield gap model, which would otherwise remain inefficient. The diet lever increases or decreases the proportion of calories derived from meat, and thus the additional calories required for animal feed. The food waste lever adjusts the calories demanded to account for waste in the food supply system. Larger values of food waste require higher cropland calories to meet the consumed calorie demands. Waste water treatment levels are modeled using per capita GDP. Therefore, the wastewater treatment lever increases per capita GDP to shift wastewater treatment systems towards higher levels of nitrogen and phosphorus removal.

We used seven basic scenarios that differed only in GDP and Population, and several scenarios to compare the effects of nutrient related levers. Business As Usual (BAU) scenarios assumed economic growth and technological improvements continued on pace with recent trends, resulting in a seven fold increase in global GDP. In contrast to BAU, in Everyone Estonia (EE) scenarios, we start with the 2005 global per capita GDP which is approximately the same as the per capita GDP of Estonia (about \$7000). We then grow the world economy at 2% annually, approximately doubling world per capita GDP and nearly quadrupling world GDP in 2050 assuming medium population growth. We assign all countries the new Estonia like per capita GDP for 2050. We combine high (10.9 billion), medium (9.6 billion) and low (8.3 billion) fertility population projections from the UN with the GDP projections for the BAU (\$260 trillion) and EE (\$149 trillion) scenarios. We also ran a nowcast scenario, which forecasts to 2050 using current values for population (6.9 billion) and GDP (\$65 trillion). The nowcast is the closest we have to a validation scenario and is used for the model validation here. The remaining

scenarios differed only in a single nutrient related lever, except the superag scenario, which combined all agriculture improvement levers into one scenario (Table 4.2).

## Validation

To validate our nitrogen yield model we compared our results with measured dissolved inorganic nitrogen and dissolved organic nitrogen values from 51 rivers around the world (compiled by Mayorga et al. 2010). We estimated total nitrogen by adding available measurements of dissolved organic nitrogen to measurements of dissolved inorganic nitrogen. The model consistently over-predicted total nitrogen yields by about 2.5 time the measured total nitrogen yields ( $R^2 = 0.52$ , Figure 4.3).

## Results

### Visions of the future

Across all scenarios, the global burden of nitrogen increased from the current conditions scenario (Figure 4.4). Both population and wealth play roles in the increased nitrogen yield we modeled. All else equal, a 14.5% increase in population from the low to medium population scenario resulted in a  $250 \text{ kg N km}^{-2} \text{ yr}^{-1}$  increase in global nitrogen yield, whereas the 75% increase in global wealth between Everyone Estonia and BAU increased global nitrogen yield by just  $168 \text{ kg N km}^{-2} \text{ yr}^{-1}$ . All of the agriculture lever scenarios based on the BAU medium population scenario increased global nitrogen yield by at least 15% from current conditions.

### Effects of nitrogen related levers

To compare potential levers to reduce the intensity of nitrogen pollution, we ran a set of scenarios varying the Genetic Gains, Nutrient Efficiency, Diet, Food Waste and Wastewater Treatment levers one at a time in relation to the Business as Usual scenario (Table 4.3). All levers reduced global nitrogen yields, but the effect was typically small (1-2% reduction, Figure 4.5). The All Ag scenario combined all of the agriculture levers

and resulted in a 6.5% reduction in global nitrogen yields. Reduction in cropland area through various levers caused the largest reductions in nitrogen yield (Table 4.6).

Reducing food waste from 30% to 15% reduced global calorie demand by 18%. This had the largest effect of all the levers tested, reducing nitrogen yields by 2.2% overall, as much as 2.7% in Africa and Latin America, and as little as 1.6% in Asia. The reduction in food waste also decreased the land required for agriculture by 0.43%, second only to improved genetics (0.58%) in reducing cropland area. Reducing food waste decreased cropland area in Africa by 0.68%, the largest of any region, and decreased demand for high fertilizer application rates (Table 4.6). Reducing food waste had the largest relative impact on agricultural nitrogen fixation (-17%) and fertilizer application (-16%), but agricultural fixation and fertilizer application contributed only about 19% to the global nitrogen yield. Shifting land out of agriculture slightly increased nitrogen yield due to natural ecosystem fixation by 4% (Table 4.3).

Genetic improvements of 25% caused the second largest decline in global nitrogen yield of 1.8%. Genetic improvements increase yields without increasing nutrient or water requirements. Increased yield through genetic improvements also reduced cropland area by 0.6% (41902 km<sup>2</sup>) which lowered fertilizer application by 15.5% globally, reduced agricultural BNF by 12% and increased ecosystem BNF by 3.7% in areas that were formerly cropland. Genetic improvements were more effective in some regions than others at reducing nitrogen yield. The differences among regions appear to be the result of differences in cropland areas the relative importance of fertilizer and agricultural biological nitrogen fixation for total nitrogen yield. Nitrogen yield reductions were as high as 2.2% in Latin America and 2.1% in Africa (where there were 0.43% and 0.84% reductions in cropland area respectively), and as low as 1.3% in Asia (0.82% cropland area reduction). The low effect in Asia is probably due to the combination of high rates of over-fertilization in existing croplands and a large proportion of the overall nitrogen budget determined by atmospheric nitrogen deposition (Figure 4.7).

The wastewater treatment lever works by increasing the per capita GDP of countries, which increases the nitrogen removal rates of treatment as determined by our wastewater treatment regressions (Figure 4.6). Increasing per capita GDP by 50% decreased global nitrogen yield by 1.4%, entirely due to a 12% reduction in the contribution of wastewater itself to the nitrogen yield. The wastewater treatment lever was most effective in the

relatively poorer and more populous continents of Africa and Asia where it reduced nitrogen yield by 4.3 and 2.1% respectively. Wastewater treatment was comparably ineffective on wealthier continents like North America (0%) and Oceania (0.1% nitrogen yield decline). Wastewater treatment has no effect on agricultural land use or yields and did not affect cropland area.

Precision agriculture resulted in a 1.2% decline in global nitrogen yield. The effects were largest in North America (2%) and Asia (1.9%). Precision agriculture had essentially no impact on nitrogen yield in Africa (0.03%). The precision agriculture lever reduces fertilizer application in areas that are above the requirements of our yield model. Since the increased nutrient efficiency doesn't increase yields, there was no change in cropland area with this lever (Tables 4.4 & 4.6). North America and Asia saw the largest declines in nitrogen yield due to decreased fertilizer use (2%), with Europe third at 1%. Latin America, Oceania and Africa saw the lowest declines at 0.4%, 0.3% and no change, respectively.

The diet lever scales the proportion of calories demanded from meat. Reducing meat consumption by 15% resulted in only 0.6% less global nitrogen yield, the smallest decline of any lever. Diet had a surprisingly small effect on cropland area, freeing only 147 km<sup>2</sup> (0.002%). Despite the small effect on cropland area, nitrogen yield due to fertilizer and agricultural nitrogen fixation declined by 4.8 and 6.6% respectively (Table 4.3). Nitrogen yield due to ecosystem fixation increased slightly (1.8%) in the land reclaimed from agriculture through diet adjustments.

All of the agriculture related levers combined (genetics, food waste, diet and precision agriculture) decreased global nitrogen yield by 6.5%, which was 14% greater than the combination of individually applied levers (5.7%). We found an even larger non-linear affect in cropland area. The combined agriculture scenario reduced cropland area by 935270 km<sup>2</sup> (13% less than BAU), 12.8 times greater than the reduced area from the individual levers combined (72968 km<sup>2</sup>). Individual levers reduced land use from the BAU scenario by 0.4% (Food Waste), 0.6% (Genetics), 0.002% (Diet), or not at all (Wastewater Treatment, Precision Ag, Table 4.5). The combined agriculture scenario resulted in the largest reductions in cropland area from BAU in Africa (122000 km<sup>2</sup>) and Asia (568000 km<sup>2</sup>, Table 4.6), both regions where expansion into new marginal croplands was required to meet additional calorie demands for food and fuels.

As shown above, nitrogen yields and the effects of the agriculture related levers varied across continents (Figure 4.7). Additionally, the major sources of nitrogen varied by continent (Table 4.7). For example, atmospheric deposition was larger in Asia than all sources combined in North America (Figure 4.7). Pasture fertilization usually contributed a small fraction to the total nitrogen source ( $<10\%$ ,  $<0.5\%$  in Africa), except in Europe (35.5% of total). Ecosystem nitrogen fixation was typically the largest single contributor to nitrogen yield, except in Asia and Europe. In Asia the largest source was atmospheric deposition, while in Europe pastures contributed the most. The contribution of wastewater to nitrogen yield in Africa (24.5%) is nearly double that of Asia (13.5%), which is again nearly double that of the next highest continent, Europe (7.4%).

### **Local sensitivity to levers**

The sensitivity to the levers varied across continents (Figure 4.8). The highest variance of nitrogen yield across scenarios for total sources was Asia, which had the highest variances for all sources except ecosystem nitrogen fixation in Latin America. The levers had especially strong effects on fertilizer and agricultural nitrogen fixation as sources of nitrogen yield in Asia. Latin America saw the second highest variance of total sources of nitrogen yield across scenarios. Nitrogen yield from agricultural fixation hand-in-hand with ecosystem fixation and fertilizers varied to similar degrees across the scenarios in Latin America. Fertilizer sources of nitrogen yield were sensitive to levers in Europe and North America. In contrast to Asia, Europe, Latin America and North America, Oceania had very low sensitivities to the levers. Africa on the other hand had the second highest sensitivity of wastewater sources to the levers.

## **Discussion**

Although strictly speaking, the future is unpredictable, we anticipate increased nitrogen yield, with the effects concentrated in places of large population growth and lower income such as Africa and Asia. These effects should be fairly robust barring events that caused large changes in population density. The effects of population touch all



aspects of the model: consumption of goods and services, demand for food, energy, water and widespread changes in land-use. Some areas were more sensitive to differences among scenarios than others. For example, nitrogen yield in Asia responded well to changes in fertilization rates through precision agriculture because current nitrogen application rates are well in excess of crop requirements in parts of China. Lowering food waste reduced cropland demand in Asia which reduced agricultural nitrogen fixation. In contrast to Asia, nitrogen yield in Africa was relatively insensitive to the choice of levers, and responded most to levers that reduced the total land in agriculture, reducing fertilizer requirements to meet crop yield goals.

The levers we tested were mildly effective at reducing nitrogen yield globally, but declines were typically less than 2%. Even the combined application of multiple levers could not maintain, much less improve upon, present day nitrogen yield. Surprisingly, although the effect of reducing food waste from 30% to 15% was singly the most effective lever, it resulted in only about a 2% reduction in global nitrogen yield. Reducing waste by half decreased calorie demand by only 18%, which while significant, paled in comparison to the caloric demands of an additional 2.6 billion people (38% more). However, the combined application of multiple levers had non-linear effects and an overall reduction in nitrogen yield of 6.5%, 14% greater than the effects of the levers applied singly. These non-additive effects appear to arise from preventing the expansion of agriculture into increasingly marginal lands with low yields and high nutrient requirements.

In most respects our results align well with results from other forecasts and integrated assessment models like IMAGE (Bouwman et al. 2006) and GlobalNEWS2 (Bouwman et al. 2009, Seitzinger et al. 2010). We found a larger nitrogen yield response to population increases than wealth increases, consistent with expectations for 12% increase in per capita food consumption (International Assessment of Agricultural Knowledge (IAASTD) 2009) and a 38% increase in population (UN 2013). Our model also identified Asia and Latin America as hotspots of nitrogen yield (Mayorga et al. 2010). Future nitrogen yield varied dramatically across continents and by sources, as expected from previous research (Seitzinger et al. 2002, Green et al. 2004, Seitzinger et al. 2005).

Very few studies have attempted to quantify the potential for nitrogen related levers to reduce nitrogen yield without reducing crop yields. Foley and others (2011) recommend four strategies for meeting cropland production goals for 2050, without overly

harming ecosystems. We implemented three of those strategies here: closing yield gaps, increasing the efficiency of nutrient fertilization, and decreasing demand through reducing food waste and meat consumption. While all of these approaches reduced nitrogen yield without sacrificing the caloric demands of the population, we were unable to maintain or reduce current nitrogen yield under future scenarios. The most promising levers (reducing food waste and meat consumption) were most effective because they reduced cropland area and thereby reduced fertilizer requirements and the spatial extent of nitrogen fixing crops. One study implemented a dietary lever similar to ours and found that reducing meat consumption could reduce fertilizer requirements and nitrogen export in North America and Europe (Seitzinger et al. 2002). Although Seitzinger and others (2002) only analyzed their diet scenario in North America and Europe, our diet lever had the lowest effect in North America and Europe, and the largest effect in Africa and Latin America (Table 4.4). We attribute this effect to the decrease in new cropland area caused by the reduced calorie demand. As a general rule, preventing agricultural expansion into marginally productive lands reduced nitrogen requirements to meet crop yields and reduced nitrogen yield.

Our model overestimates nitrogen yields of rivers by about 2.5 times measured yields. There are two potential sources for this error, we either overestimate nitrogen inputs to the nitrogen balance, or underestimate the effects of nitrogen retention. One aspect in particular of our nitrogen balance model may have contributed to an overestimation of nitrogen inputs. We used middle-of-the-range nitrogen fixation rates from Cleveland and others (1999), which were identified as the best available estimate of ecosystem nitrogen fixation in a recent review (Sobota et al. 2013). However, a newer approach based on stable isotopes and mass balance (Vitousek et al. 2013) reduced estimates nitrogen fixation rates to levels similar to the low-area fixation rates from Cleveland and others (1999, Bouwman et al. 2013).

On the retention side, we use a simple runoff based nitrogen retention function adapted from GlobalNEWS2 (Mayorga et al. 2010) to calculate basin-wide nitrogen retention. The retention function coefficients from GlobalNEWS2 are based on a global calibration with measured river nitrogen yields, and implicitly include the effects of lakes and wetlands within the drainage basins they were calibrated over (Mayorga et al. 2010). The GlobalNEWS2 retention coefficients do not include coefficients for total

nitrogen, which is the form of nitrogen we track. We used the simplest approach and used the retention coefficients for dissolved inorganic nitrogen from general and agricultural areas. This probably contributed to our overestimation of nitrogen yields by failing to differentiate between the humid tropics and general and agricultural areas. The maximum export of DIN from general and agricultural areas (97.75%) is about nine times greater than the export from the humid tropics (11%). The humid tropics account for approximately 20% of the terrestrial surface, so reducing their losses by a factor of nine would lower global export by a factor of 1.78. This degree of nitrogen export overestimation would account for a majority of our model overestimation. Another source of overestimation caused by our choice of nitrogen retention coefficients is that the maximum export of inorganic nitrogen (97.75%) is almost two orders of magnitude greater than organic nitrogen (1%). The proportion of inorganic and organic nitrogen varies in streams and depends in part on agriculture and other sources of nitrogen pollution (Perakis and Hedin 2002) making it difficult to estimate the magnitude of overestimation this may cause. However, increased nitrogen deposition and agriculture in 2050 will result in more pervasive nitrogen pollution and will probably result in stream fluxes dominated by inorganic forms. More areas of predominantly inorganic nitrogen export would reduce the importance of higher organic nitrogen retention rates on our model overestimation.

Both sources of error, overestimates of nitrogen balance and underestimated nitrogen retention, could result in incorrect conclusions from our model. Estimation errors of one or more components of the nitrogen balance could result in over or underestimation of the effectiveness of a particular lever. The potential for overestimation of nitrogen fixation in our model is distributed equally across the globe, e.g, nitrogen fixation rates are over-estimated for all ecosystems. Because the distribution of these errors is unbiased, the overestimation biases should not affect the comparison of effectiveness of the levers among the regions.

Our simple nitrogen retention scheme could result in model interpretation errors if the errors in retention vary by regions. The omission of lakes and wetlands could bias the model regionally due to differences in the density of these aquatic features. Sadly, the global decline in natural wetlands over the last century will probably continue as wetlands are drained for agricultural expansion, the primary driver of wetland loss (Spiers

2001). The agricultural intensification and expansion required meet the need for food and fuel in our model could potentially homogenize the differences in wetland density among regions and reduce the effectiveness of wetland nitrogen retention. Moreover, the global calibration of the retention coefficients implicitly includes the effects of lakes and wetlands, which are distributed fairly evenly across continents (Spiers 2001), suggesting that our comparisons of levers among regions should be robust.

Although there are potential hazards to our simplified nitrogen retention scheme, the nitrogen yields reported here nevertheless reflect the differences in net total nitrogen inputs between regions. Although climate plays an important role in modulating riverine nitrogen fluxes on annual and sub-annual time scales (Donner and Scavia 2007, Kane et al. 2008, Howarth et al. 2012), net total nitrogen inputs are the dominant driver of nitrogen fluxes despite wide variability in climate and other variables thought to be important to nitrogen retention and fluxes (Howarth et al. 2012, Baron et al. 2013). Our results should therefore accurately portray the essential differences in nitrogen yields among continents. Furthermore, by aggregating results to the continental scale model errors associated with differences in nitrogen retention should cancel out each-other.

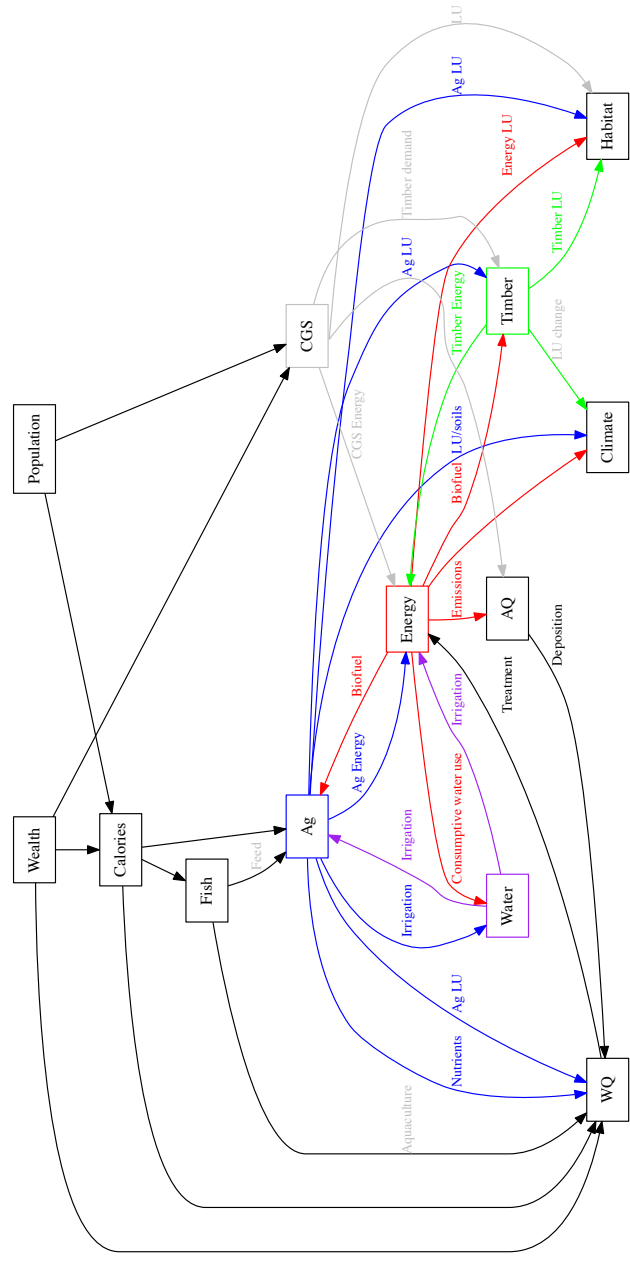
The other major limitation of this study is the limited range of technological levers with which to transform the future. We chose to implement what we believed were the levers with the greatest potential to reduce nitrogen yields given today's technology, and given strategies outlined by others (Foley et al. 2011). However, unforeseen technologies, changes in governance, human culture and climatic extremes could and probably will shape the future of nitrogen dynamics. Given the unpredictable nature of these things, we chose instead to focus on the potential gains from current technologies. Our results suggest that strategies that reduce the need to expand into increasingly marginal cropland in the future yield the highest reductions in nitrogen yield. These conclusions should be robust to the uncertainties in population, wealth and diet forecasts as long as marginal croplands continue to provide lower crop yields and require higher nutrient inputs.

## **Conclusion**

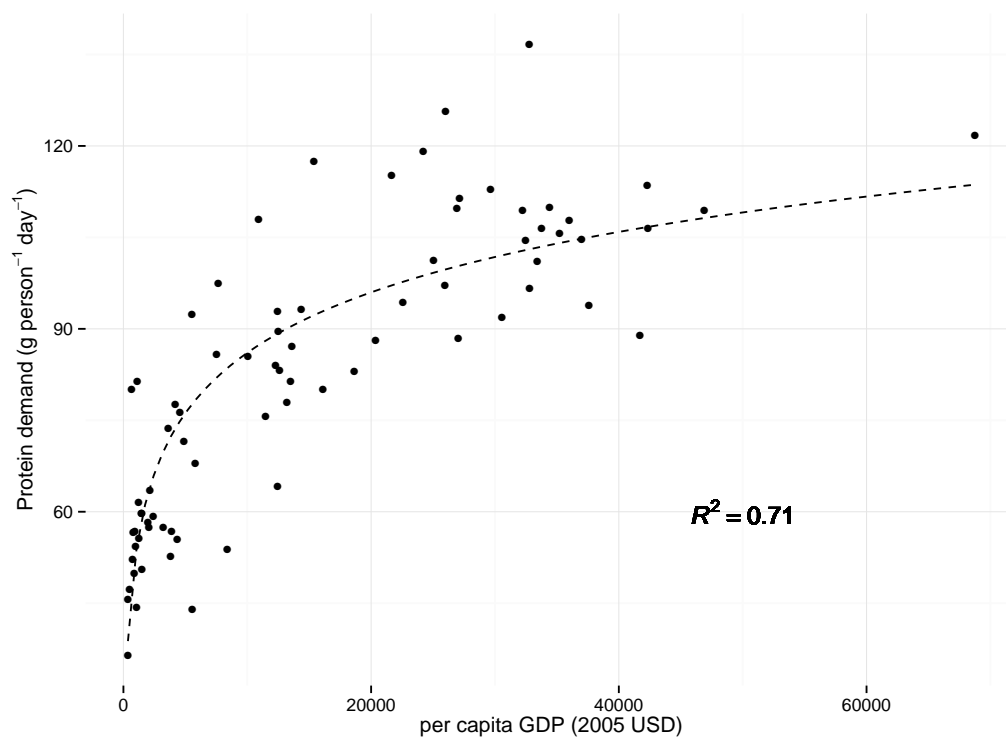
Not all levers were effective everywhere, yet providing a reasonable quality of life will require reducing nitrogen pressures on ecosystems everywhere. Africa, Asia and North

America responded differently to the levers, as expected from current differences in agricultural nutrient balances (Vitousek et al. 2009). In Africa, reducing the footprint of agriculture was the most effective way to reduce nitrogen yield where most agriculture suffers from low soil fertility. In contrast, increasing the efficiency of nitrogen application was particularly effective in Asia where national policies supporting fertilizer use have resulted in large nitrogen excesses. As illustrated by the combined agriculture scenario, synergistic effects exist among levers. These synergistic effects were the result of preventing the expansion of agriculture into increasingly marginal land, which not only reduced the spatial extent of nitrogen applications, but also removed areas with the highest nitrogen application requirements from cropland. Efforts to address nitrogen pollution should increase the options available (levers) for reducing nitrogen demand and increasing the efficiency of nitrogen use (Godfray et al. 2010). Some levers may be better suited to one climate or terrain than others, and novel combinations of levers may work better in some areas than others. Despite causing large reductions in cropland area in Asia, many levers were relatively ineffective there because of the high proportion of reactive nitrogen from atmospheric deposition. This highlights the importance of reducing all sources of nitrogen and not focusing solely on cropland sources (Galloway and Cowling 2002). While attractive from a public policy perspective, global levers may not work well in some areas, and top down efforts often experience policy resistance at the level of local implementation due to poor understanding of the feedbacks in the local system (Sterman 2000). For food and nutrient systems to be most effective they should be developed specific to localities and encourage a diversity of crop choices and nutrient management levers (Kearney 2010).

## Figures and Tables

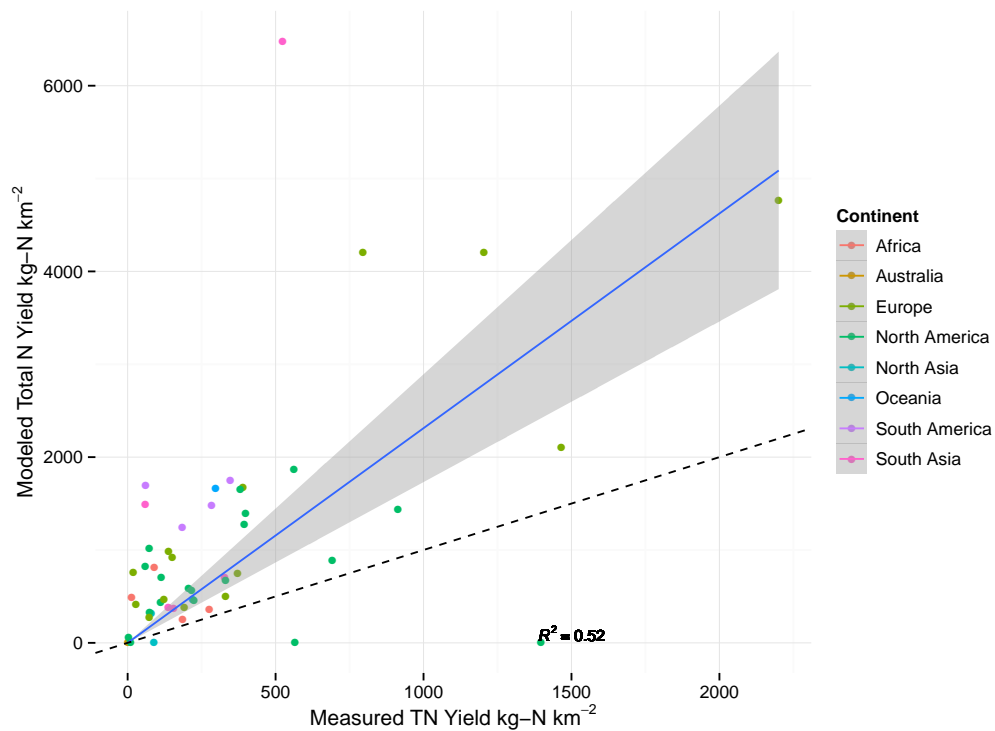


**Figure 4.1** The Make it Fit model framework. Lines indicate connections between modules. AQ: air quality, CGS: consumer goods and services, LU: land-use, WQ: water quality.

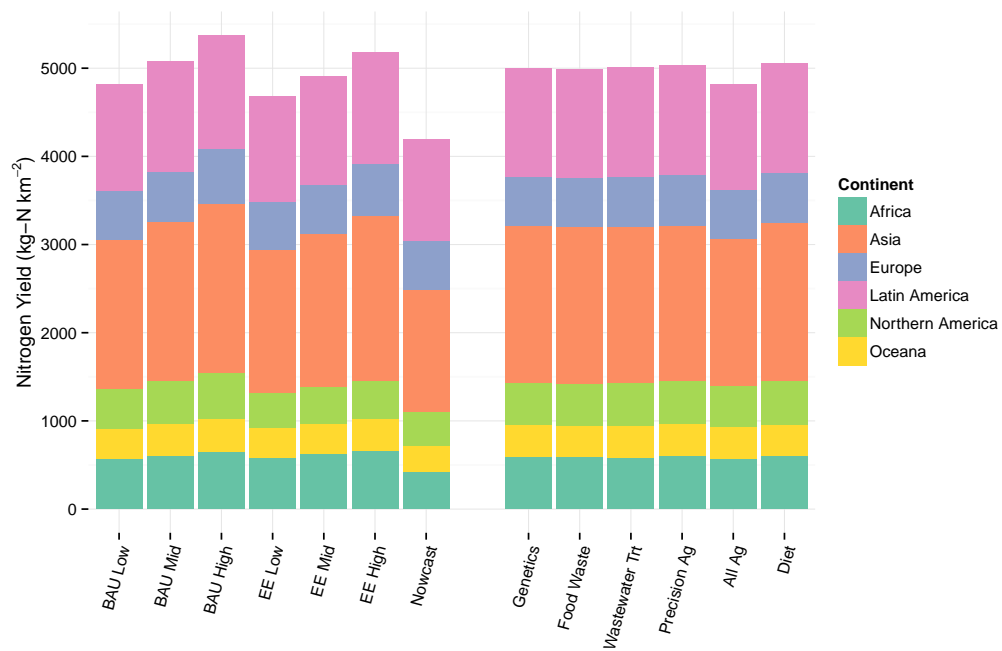


**Figure 4.2** Relationship between per capita protein demand (UN FAO) and per capita GDP (PriceWaterhouse Cooper). Dashed line indicates fit of  $\log(\text{per capita GDP})$  to protein demand.

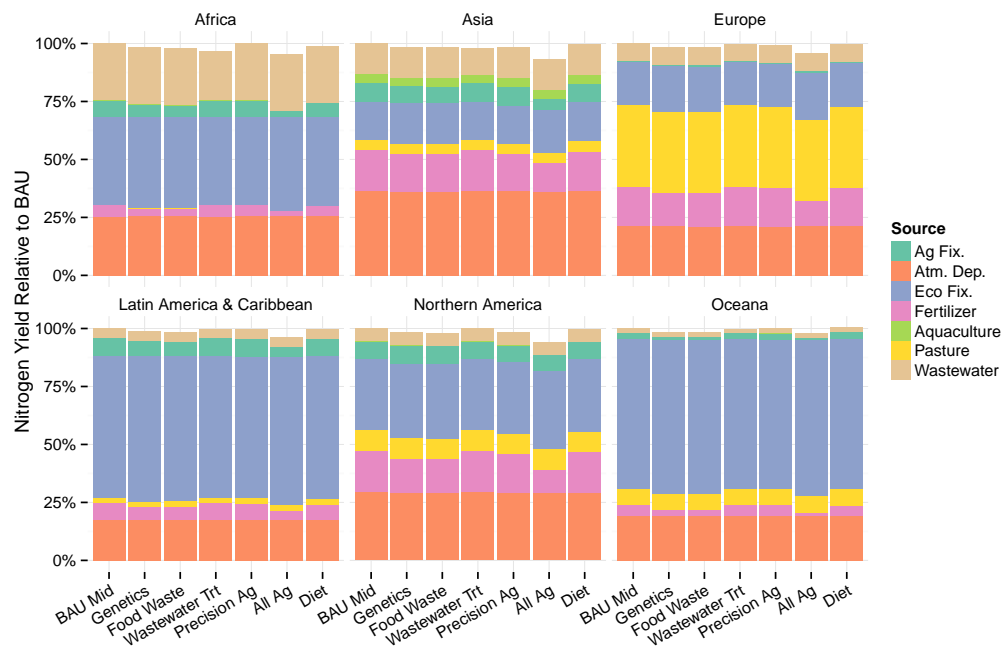




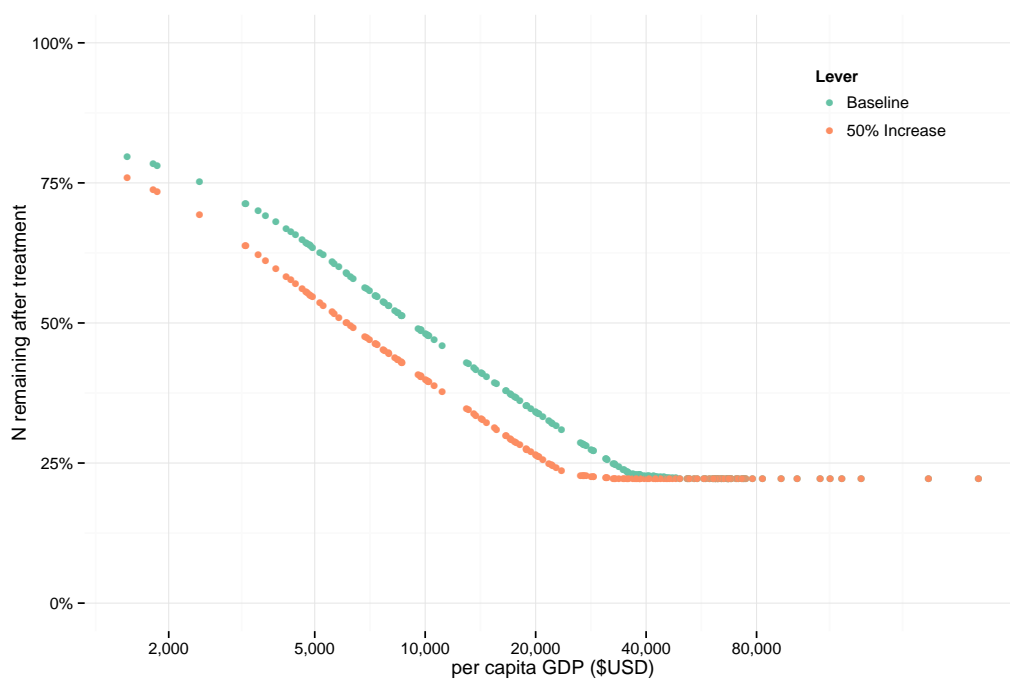
**Figure 4.3** Measured riverine dissolved nitrogen yields ( $\text{kg N km}^{-2} \text{ yr}^{-1}$ ) compared to nowcast scenario modeled yields. Each point is a river with measured and modeled yields. Solid line and shading indicate least squares fit with intercept forced through origin and standard error, dashed line indicates 1:1 relationship.



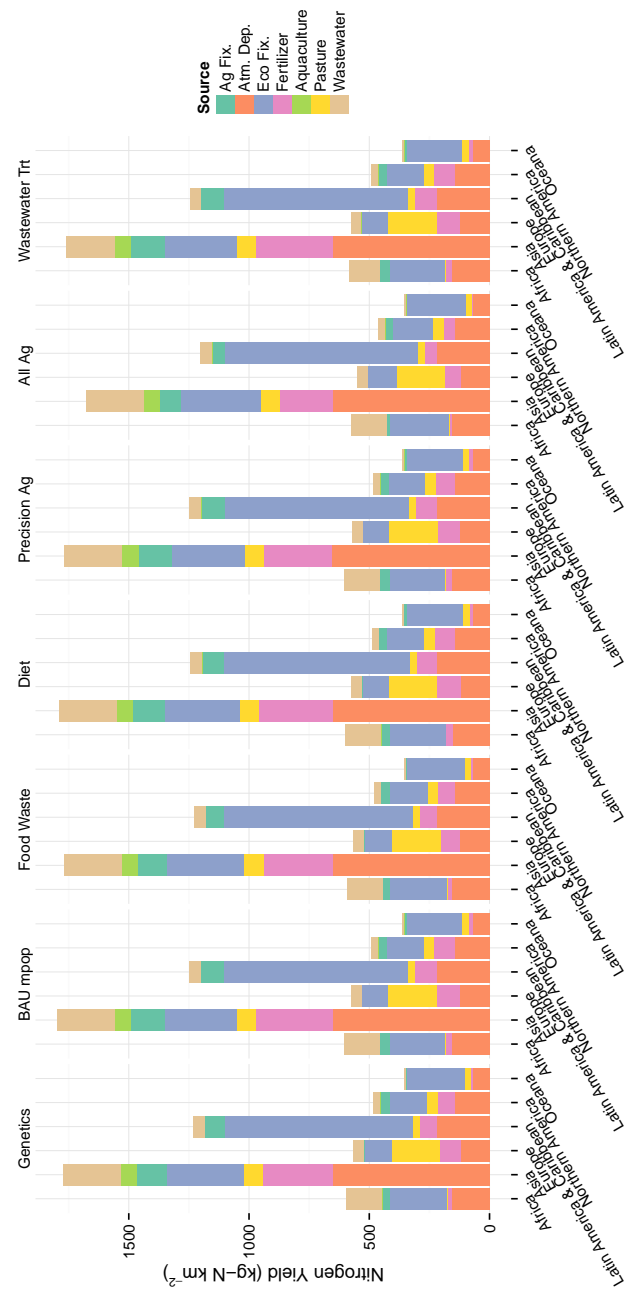
**Figure 4.4** Global nitrogen yield for different scenarios of nitrogen reduction strategies



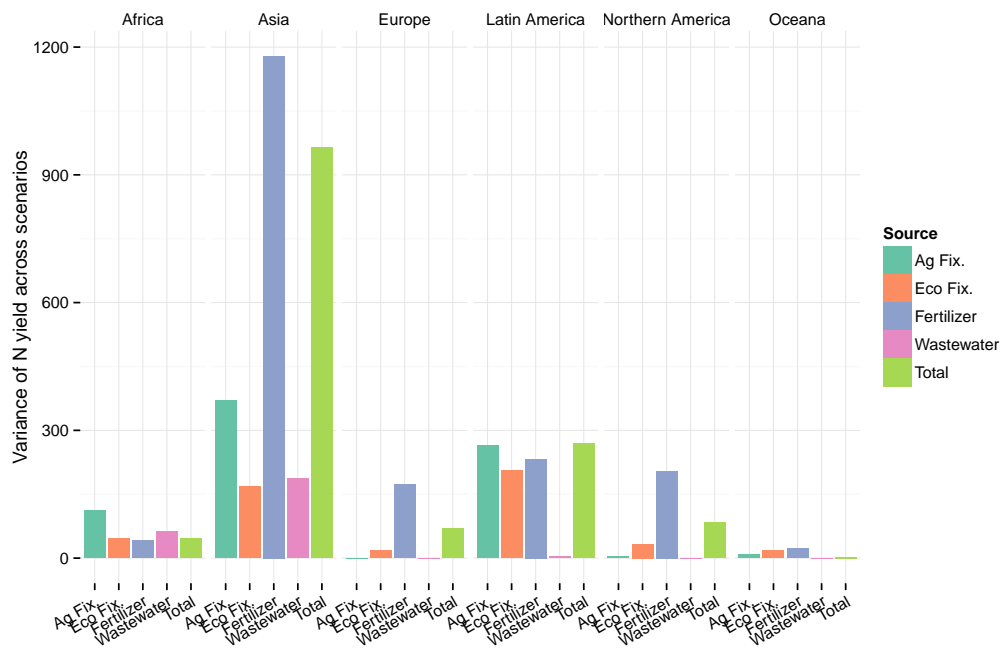
**Figure 4.5** Nitrogen yield relative to baseline (BAU Mid) for different scenarios of nitrogen reduction strategies



**Figure 4.6** Effect of increasing per capita GDP (50% increase) on wastewater treatment nitrogen removal



**Figure 4.7** Nitrogen yield across continents and by source of nitrogen under different scenarios of nitrogen reduction strategies. See 4.2 for an explanation of scenarios. Ag Fix.: agricultural biological nitrogen fixation, Atm. Dep.: atmospheric nitrogen deposition, Eco Fix.: ecosystem biological nitrogen fixation.



**Figure 4.8** Variance of nitrogen yield across the agriculture and wastewater scenarios

**Table 4.1** Summary of Make it Fit Framework modules.

Module	Inputs/Drivers	Outputs	Method
Calorie Demand	Population, GDP, food waste, meat in diet	Calories demanded for food crops, livestock, fish and feed crops	Regression models
Goods and Services Consumption	Population, GDP	Demand for energy and natural resources from industry, construction, services, residential and transportation sectors	Regression models
Aquaculture and Fisheries	Fisheries sustainability scenario, fish calorie demand	Energy and aquaculture feed demand, fleet and operations emissions	Balance sheets (Max Troell and Marc Metian, personal communication, 2013) and fisheries productivity models (Costello et al. 2012, and Ricard et al. 2012)
Cropland Agriculture	Food, feed and fuel calories demanded, land cover and use, nutrient use efficiency, land use efficiency, yield gap goals for intensified and expanded agriculture	Area cultivated, calories delivered, nitrogen, phosphorus and water use	Yield gap model (Mueller et al. 2012)

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Module	Inputs/Drivers	Outputs	Method
Pasture	Land cover and use	Loss of pasture area	Land balance
Energy	Demand from all other modules	Liquid, head and electric energy production, resources used, atmospheric emissions, consumptive water use	Constrained optimization
Timber	Demand for roundwood and fuelwood, land cover and use	Land cover and use	Balance model
Climate	Land cover change, emissions from other modules	Atmospheric CO <sub>2</sub>	Box model
Air Quality	Intensity of economic sectors	NO <sub>x</sub> , SO <sub>2</sub> and PM <sub>10</sub> emissions	Linear scaling functions
Water Quantity	Runoff, evapotranspiration, irrigation and energy consumptive water use	water balance and flow	Mass balance, routing model



**Table 4.2** Scenario definitions, baseline scenario *BAU Med* in *italics*, variations from baseline in **bold**

Scenario	GDP <sup>a</sup>	Population <sup>b</sup>	Genetic Gains <sup>c</sup>	Precision Ag <sup>d</sup>	Diet <sup>e</sup>	Food Waste <sup>f</sup>	Wastewater <sup>g</sup>
BAU High	260	<b>10.9</b>	1	off	1	30%	1
<i>BAU Med</i>	<i>260</i>	<i>9.6</i>	<i>1</i>	<i>off</i>	<i>1</i>	<i>30%</i>	<i>1</i>
BAU Low	260	<b>8.3</b>	1	off	1	30%	1
EE Med	149	9.6	1	off	1	30%	1
EE Low	149	<b>8.3</b>	1	off	1	30%	1
EE High	149	<b>10.9</b>	1	off	1	30%	1
Nowcast	65	<b>6.9</b>	1	off	1	30%	1
Genetic Gains	260	9.6	<b>1.25</b>	off	1	30%	1
Precision Ag	260	9.6	1	<b>on</b>	1	30%	1
Diet	260	9.6	1	off	<b>0.85</b>	30%	1
Food Waste	260	9.6	1	off	1	<b>15%</b>	1
Wastewater	260	9.6	1	off	1	30%	<b>1.5</b>
All Ag	260	9.6	<b>1.25</b>	<b>on</b>	<b>0.85</b>	<b>15%</b>	1

<sup>a</sup> Trillion USD, <sup>b</sup> Billions <sup>c</sup> Proportional change in crop yield <sup>d</sup> Precision Ag: when on, limits nitrogen fertilizer application rates to the minimum required to meet yield goals predicted by the agriculture submodel. When off, nitrogen fertilizer is applied at current rates in places where it is currently over-applied. <sup>e</sup> The percentage of meat demanded in diets from the baseline scenario. <sup>f</sup> Food Waste is the percentage of calories that do not contribute to human nutrition due to waste. <sup>g</sup> Wastewater Treatment is the proportional change in per capita GDP used to predict the mix of wastewater treatment plants and their nitrogen removal efficiency.

**Table 4.3** Percent change in nitrogen yield ( $\text{kg-N km}^{-2} \text{ year}^{-1}$ ) relative to BAU Medium Population

Scenario	Ag Fix. <sup>a</sup>	Atm. Dep. <sup>b</sup>	Eco Fix. <sup>c</sup>	Fertilizer	Aquaculture	Pasture	Wastewater	Total
Genetics	-12.01	-0.00	3.65	-15.52	0.13	0.00	0.00	-1.76
Food Waste	-17.19	-0.00	4.11	-16.32	0.15	0.00	0.00	-2.15
Diet	-6.55	0.00	1.78	-4.81	0.05	0.00	0.00	-0.58
Precision Ag	0.00	0.00	0.00	-7.35	0.00	0.00	0.00	-1.22
All Ag	-41.39	-0.01	7.05	-38.32	0.27	0.00	0.00	-6.51
Wastewater Trt	0.00	0.00	0.00	0.00	0.00	0.00	-12.37	-1.42

<sup>a</sup> Agricultural nitrogen fixation. <sup>b</sup> Atmospheric nitrogen deposition. <sup>c</sup> Non agricultural ecosystem nitrogen fixation.

**Table 4.4** Percent change in nitrogen yield ( $\text{kg-N km}^{-2} \text{ year}^{-1}$ ) relative to BAU Medium Population

scenario	Africa	Asia	Europe	Latin America	Northern America	Oceania
Genetics	-2.06	-1.31	-1.98	-2.20	-2.00	-1.59
Food Waste	-2.68	-1.62	-2.19	-2.69	-2.54	-1.85
Diet	-1.27	-0.52	-0.38	-0.80	-0.13	-0.12
Precision Ag	-0.03	-1.94	-0.92	-0.40	-2.03	-0.31
All Ag	-5.89	-7.21	-5.25	-6.22	-7.49	-3.89
Wastewater Trt	-4.33	-2.13	-0.24	-0.55	-0.00	-0.10

**Table 4.5** Global cropland areas by scenario (km<sup>2</sup>) and percent change from BAU

Scenario	Area (km <sup>2</sup> )	$\Delta$ Area (km <sup>2</sup> )	%Change
Genetics	7169144	-41902	-0.58
BAU Mid	7211046	0	0.00
Food Waste	7179994	-31052	-0.43
Diet	7210899	-147	-0.00
Precision Ag	7211046	0	0.00
All Ag	6275776	-935270	-12.97
Wastewater Trt	7211046	0	0.00

**Table 4.6** Continental cropland areas by scenario (km<sup>2</sup>) and percent change from BAU Mid

Continent	Scenario	Area (km <sup>2</sup> )	$\Delta$ Area (km <sup>2</sup> )	%Change
Africa	BAU Mid	565079.55	0.00	0.00
Africa	Genetics	560340.75	-4738.80	-0.84
Africa	Food Waste	561248.55	-3831.00	-0.68
Africa	Diet	565053.14	-26.41	-0.00
Africa	Precision Ag	565079.55	0.00	0.00
Africa	Wastewater Trt	565079.55	0.00	0.00
Africa	All Ag	442919.21	-122160.34	-21.62
Oceania	BAU Mid	184923.57	0.00	0.00
Oceania	Genetics	184481.87	-441.70	-0.24
Oceania	Food Waste	184496.05	-427.51	-0.23
Oceania	Diet	184918.61	-4.95	-0.00
Oceania	Precision Ag	184923.57	0.00	0.00
Oceania	Wastewater Trt	184923.57	0.00	0.00
Oceania	All Ag	119186.29	-65737.28	-35.55
Northern America	BAU Mid	1083422.43	0.00	0.00
Northern America	Genetics	1082492.51	-929.92	-0.09
Northern America	Food Waste	1082803.44	-618.99	-0.06
Northern America	Diet	1083403.55	-18.88	-0.00
Northern America	Precision Ag	1083422.43	0.00	0.00
Northern America	Wastewater Trt	1083422.43	0.00	0.00
Northern America	All Ag	1056174.85	-27247.58	-2.51
Asia	BAU Mid	3369911.64	0.00	0.00
Asia	Genetics	3342206.77	-27704.87	-0.82
Asia	Food Waste	3350206.27	-19705.36	-0.58
Asia	Diet	3369867.56	-44.07	-0.00

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Continent	Scenario	Area (km <sup>2</sup> )	$\Delta$ Area (km <sup>2</sup> )	%Change
Asia	Precision Ag	3369911.64	0.00	0.00
Asia	Wastewater Trt	3369911.64	0.00	0.00
Asia	All Ag	2801291.18	-568620.45	-16.87
Europe	BAU Mid	1242170.83	0.00	0.00
Europe	Genetics	1237788.87	-4381.96	-0.35
Europe	Food Waste	1239004.24	-3166.59	-0.25
Europe	Diet	1242146.92	-23.91	-0.00
Europe	Precision Ag	1242170.83	0.00	0.00
Europe	Wastewater Trt	1242170.83	0.00	0.00
Europe	All Ag	1181953.03	-60217.80	-4.85
Latin America	BAU Mid	756278.25	0.00	0.00
Latin America	Genetics	753053.18	-3225.07	-0.43
Latin America	Food Waste	753423.56	-2854.69	-0.38
Latin America	Diet	756243.76	-34.50	-0.00
Latin America	Precision Ag	756278.25	0.00	0.00
Latin America	Wastewater Trt	756278.25	0.00	0.00
Latin America	All Ag	674709.36	-81568.90	-10.79

**Table 4.7** Percent of total nitrogen yield attributed to source

Scenario	Source	Africa	Asia	Europe	Latin America	N. America	Oceania
Genetics	Atm. Dep.	26.04	36.83	21.58	17.81	29.76	19.51
Genetics	Fertilizer	3.31	16.26	14.80	5.74	14.69	2.47
Genetics	Pasture	0.41	4.53	35.54	2.37	9.10	7.41
Genetics	Eco Fix.	39.67	17.90	19.74	63.48	32.70	67.13
Genetics	Ag Fix.	5.59	7.21	0.79	6.61	7.99	1.64
Genetics	Aquaculture	0.07	3.78	0.00	0.00	0.23	0.00
Genetics	Wastewater	24.91	13.49	7.54	3.98	5.55	1.83
BAU Mid	Atm. Dep.	25.60	36.33	21.22	17.57	29.30	19.23
BAU Mid	Fertilizer	4.75	17.67	17.23	7.29	18.08	4.63
BAU Mid	Pasture	0.40	4.47	34.95	2.34	8.96	7.30
BAU Mid	Eco Fix.	37.55	16.65	18.52	61.06	30.72	64.34
BAU Mid	Ag Fix.	7.16	7.84	0.66	7.83	7.26	2.70
BAU Mid	Aquaculture	0.07	3.72	0.00	0.00	0.22	0.00
BAU Mid	Wastewater	24.48	13.31	7.41	3.92	5.46	1.81
Food Waste	Atm. Dep.	26.16	36.92	21.62	17.87	29.88	19.53
Food Waste	Fertilizer	3.27	16.24	14.59	5.66	14.55	2.41
Food Waste	Pasture	0.41	4.54	35.61	2.38	9.14	7.42
Food Waste	Eco Fix.	40.10	18.07	19.87	63.89	32.99	67.33
Food Waste	Ag Fix.	4.96	6.92	0.75	6.21	7.64	1.48
Food Waste	Aquaculture	0.07	3.79	0.00	0.00	0.23	0.00
Food Waste	Wastewater	25.02	13.52	7.55	3.99	5.57	1.83
Diet	Atm. Dep.	25.84	36.49	21.29	17.65	29.35	19.16
Diet	Fertilizer	4.19	17.19	16.69	6.65	17.32	4.14
Diet	Pasture	0.41	4.49	35.07	2.35	8.97	7.28
Diet	Eco Fix.	38.78	17.21	18.84	62.21	31.36	64.88
Diet	Ag Fix.	6.00	7.51	0.67	7.20	7.31	2.73
Diet	Aquaculture	0.07	3.74	0.00	0.00	0.22	0.00
Diet	Wastewater	24.72	13.36	7.44	3.94	5.47	1.80

*continued on next page...*

Scenario	Source	Africa	Asia	Europe	Latin America	N. America	Oceania
Precision Ag	Atm. Dep.	25.61	36.98	21.39	17.61	29.77	19.25
Precision Ag	Fertilizer	4.73	16.21	16.59	7.05	16.77	4.50
Precision Ag	Pasture	0.40	4.55	35.22	2.35	9.10	7.31
Precision Ag	Eco Fix.	37.56	16.95	18.67	61.22	31.21	64.42
Precision Ag	Ag Fix.	7.16	7.98	0.67	7.84	7.37	2.71
Precision Ag	Aquaculture	0.07	3.79	0.00	0.00	0.23	0.00
Precision Ag	Wastewater	24.49	13.54	7.47	3.93	5.55	1.81
All Ag	Atm. Dep.	26.83	38.93	22.20	18.27	31.08	19.66
All Ag	Fertilizer	2.06	12.98	11.43	4.12	10.53	1.47
All Ag	Pasture	0.42	4.79	36.57	2.43	9.50	7.47
All Ag	Eco Fix.	42.48	19.84	21.37	66.64	35.89	68.72
All Ag	Ag Fix.	2.48	5.21	0.66	4.46	6.96	0.84
All Ag	Aquaculture	0.07	4.00	0.00	0.00	0.24	0.00
All Ag	Wastewater	25.66	14.26	7.76	4.08	5.79	1.85
Wastewater	Atm. Dep.	26.52	37.08	21.26	17.63	29.30	19.24
Wastewater	Fertilizer	4.92	18.04	17.27	7.31	18.08	4.63
Wastewater	Pasture	0.42	4.56	35.02	2.35	8.96	7.31
Wastewater	Eco Fix.	38.90	17.00	18.56	61.29	30.72	64.40
Wastewater	Ag Fix.	7.41	8.00	0.66	7.85	7.26	2.71
Wastewater	Aquaculture	0.07	3.80	0.00	0.00	0.22	0.00
Wastewater	Wastewater	21.76	11.53	7.22	3.57	5.46	1.71



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## Appendix A

# Daily respiration model selection

We compared one-, two- and three-pool models that predicted daily respiration, as follows.

$$C_{rate(t)} = k_f(C_f e^{-k_f t}) \quad (\text{A.1})$$

$$C_{rate(t)} = k_f(C_f e^{-k_f t}) + k_s[(C_t - C_f) e^{-k_s t}] \quad (\text{A.2})$$

$$C_{rate(t)} = k_f(C_f e^{-k_f t}) + k_s[(C_t - C_f - C_{NHC}) e^{-k_s t}] + k_r(C_{NHC} e^{-k_r t}) \quad (\text{A.3})$$

where  $C_{rate}$  is the daily respiration rate ( $\text{mg C g soil}^{-1} \text{ day}^{-1}$ ),  $C_f$  is the labile carbon pool ( $\text{mg C g soil}^{-1}$ ),  $C_{NHC}$  is nonhydrolyzable or resistant C (NHC;  $\text{mg C g soil}^{-1}$ ),  $C_t$  is total C ( $\text{mg C g soil}^{-1}$ ),  $k_f$ ,  $k_s$ , and  $k_r$  are the decomposition rates of the labile (fast), slow, and resistant pools (respectively;  $\text{day}^{-1}$ ), and  $t$  is time in days. The slow C pool ( $C_s$ ) is defined in the above equation as  $C_t - C_f - C_{NHC}$ . The mean residence time of the  $C_{NHC}$  was set at  $< 1000$  years (Paul et al. 2006; Pendall and King 2007  $k_r = 2.7 \times 10^{-6} \text{ day}^{-1}$ ). As was found by Paul et al. (2001) and Pendall and King (2007), the choice of a  $k_r$  (100–1000 years) did not influence the parameter estimates of the faster soil C pools or fluxes (Table A.1). We used Akaike's Information Criterion corrected for small sample sizes (AICc) to choose among the three models in the candidate model set (Eq. 13). AICc uses maximum likelihood to estimate the relative Kullback-Leibler distance (the amount of information lost by using a model to approximate truth) between competing models. This method determines the model

closest to the unknown truth, which is represented by the data (Burnham and Anderson 2002). The model with the lowest AICc has the most support in the data and is closest to the unknown truth. A difference in AICc points ( $\Delta\text{AICc}$ ) between two candidate models of  $> 3$  was used to indicate a significant difference in model fits. All models were fit and AICc values calculated using maximum likelihood estimation and the `bbmle` package (Bolker 2010) in the R 2.12.0 environment (R Development Core Team 2011). In the majority of cases (74/112), there was no difference between the two and three pool models ( $\Delta\text{AICc}$  of both models  $< 3$ ), while the one pool model fit the data poorly compared to the two and three pool models ( $\Delta\text{AICc} > 3$ ; Table A.2). In 31 of 112 cases all three models fit the data equally well (all  $\Delta\text{AICc} < 3$ ). In seven cases, the one pool model was the best model ( $\Delta\text{AICc} = 0$ ;  $\Delta\text{AICc}$  of the two and three pool models  $> 3$ ). We chose to focus on the parameter estimates from the three pool model for several reasons: (1) the two and three pool models better accounted for the long term dynamics of soil respiration in our incubations than did the single pool model; (2) the two and three pool models fit the data equally well and better than the one pool model in the majority of cases (Table A.2); (3) obtaining separate estimates for slow and resistant pools and fluxes allowed us to parse out the effects of BioCON treatments on each pool (versus a two pool model which lumps resistant and slow carbon into the second/slow pool) and expand upon previous work in BioCON that analyzed long-term incubations using a two-pool model (Dijkstra et al. 2005); and (4) Paul et al. (2001) found that using a two pool model (constrained by total C) substantially underestimated both the size and decomposition rate of the slow C pool. We also found that the two pool model consistently resulted in a slower  $k_s$ , but a larger Cs. Estimates of  $k_f$  and  $C_f$  were unaffected by model choice (Table A3). The three pool model explained more than 90% of the variation in daily respiration rates in 66/112 cases; between 70–90% of the variation in the data in 42/112 cases; and between 60–70% of the variation in the data in 4 cases (Table A.2).

**Table A.1** Regression parameters for linear regressions between the parameters for the three pool model using a  $k_r$  100 years ( $k_r = 0.0000274$ ) and the three pool model using a  $k_r$  1000 years ( $k_r = 0.0000027$ )

	Intercept	Slope	$r^2$
$k_f$	0.0001	1.005	1.000
$C_f$	0.0006	0.996	1.000
$k_s$	0.0000	1.020	0.999

**Table A.2** AICc model selection results and  $r^2$  for the one, two and three pool daily respiration models.  $\Delta\text{AICc}$  is the difference between the AICc score of the best model (the model with the lowest AICc value) and the other models in the set. =CO2 is ambient CO2, +CO2 is elevated CO2, =N is ambient N, +N is added N, #Spp is number of species.

No	CO <sub>2</sub>	N	#Spp	$\Delta\text{AICc}$			AICc Weights			$r^2$		
				1	2	3	1	2	3	1	2	3
1	+CO2	=N	1	3.21	0.32	0	0.098	0.415	0.487	0.534	0.732	0.739
2	=CO2	=N	1	1.47	0.03	0	0.194	0.4	0.406	0.927	0.949	0.949
3	+CO2	+N	1	17.1	0.89	0	0	0.391	0.609	0.824	0.96	0.963
4	+CO2	+N	16	0	2.61	2.6	0.648	0.176	0.177	0.684	0.714	0.714
5	+CO2	=N	1	9.75	1.04	0	0.005	0.371	0.625	0.771	0.909	0.917
6	=CO2	=N	16	12.07	0.49	0	0.001	0.439	0.56	0.878	0.954	0.956
7	=CO2	=N	1	12.33	0	0.08	0.001	0.509	0.49	0.53	0.866	0.865
8	=CO2	=N	16	7.63	0.17	0	0.011	0.473	0.515	0.831	0.912	0.913
9	+CO2	+N	1	17.69	0.29	0	0	0.464	0.536	0.469	0.899	0.901
10	+CO2	+N	16	1.28	0.07	0	0.211	0.388	0.401	0.818	0.874	0.875
11	+CO2	=N	16	8.34	0.33	0	0.008	0.456	0.536	0.592	0.841	0.846
12	+CO2	+N	16	5.66	0.95	0	0.035	0.37	0.595	0.766	0.878	0.887
13	+CO2	+N	16	0	0.59	0.55	0.4	0.298	0.303	0.944	0.956	0.956
14	=CO2	+N	1	8.57	0.38	0	0.007	0.449	0.543	0.771	0.907	0.91
15	=CO2	=N	1	10.69	0.36	0	0.003	0.454	0.543	0.731	0.909	0.912
16	+CO2	=N	1	7.92	0.21	0	0.01	0.469	0.521	0.716	0.878	0.88
17	=CO2	+N	1	10.1	0.17	0	0.003	0.477	0.52	0.75	0.905	0.906
18	=CO2	=N	16	4.92	0.27	0	0.044	0.446	0.51	0.913	0.956	0.956
19	+CO2	=N	1	6.09	0.14	0	0.024	0.471	0.505	0.735	0.876	0.878
20	+CO2	=N	1	0.7	0.26	0	0.272	0.34	0.387	0.844	0.89	0.892
21	+CO2	=N	1	2.98	0.07	0	0.103	0.441	0.456	0.756	0.856	0.857
22	=CO2	=N	16	5.24	0	0.05	0.036	0.489	0.476	0.908	0.952	0.952
23	+CO2	=N	16	8.24	0.24	0	0.009	0.466	0.526	0.78	0.913	0.914

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No	CO <sub>2</sub>	N	#Spp	1	2	3	1	2	3	1	2	3
24	=CO2	+N	1	10.87	0.12	0	0.002	0.483	0.514	0.894	0.962	0.963
25	+CO2	=N	16	0	1.24	1.19	0.478	0.258	0.264	0.918	0.933	0.933
26	=CO2	=N	16	0	2.13	2.11	0.59	0.204	0.206	0.852	0.873	0.873
27	=CO2	+N	16	7.04	0.17	0	0.015	0.471	0.514	0.894	0.952	0.953
28	+CO2	+N	1	13.23	0.43	0	0.001	0.446	0.553	0.745	0.928	0.93
29	+CO2	+N	1	0	0.47	0.46	0.387	0.306	0.307	0.81	0.85	0.85
30	=CO2	=N	16	0	4.08	4.07	0.793	0.103	0.104	0.837	0.84	0.84
31	+CO2	=N	1	8.74	0.3	0	0.007	0.46	0.533	0.699	0.885	0.887
32	=CO2	+N	16	0	3.42	3.41	0.734	0.133	0.134	0.827	0.837	0.838
33	+CO2	=N	1	3.95	0.11	0	0.067	0.454	0.48	0.854	0.918	0.919
34	+CO2	=N	16	14.02	0.2	0	0	0.475	0.525	0.806	0.946	0.947
35	+CO2	+N	16	13.19	0.29	0	0.001	0.464	0.536	0.908	0.974	0.974
36	=CO2	=N	16	2.3	0.32	0	0.146	0.393	0.461	0.83	0.893	0.896
37	=CO2	+N	1	5.27	0.08	0	0.035	0.473	0.492	0.847	0.906	0.907
38	=CO2	+N	16	0	0.57	0.51	0.396	0.298	0.306	0.873	0.901	0.902
39	=CO2	+N	1	6.4	0	0.04	0.02	0.494	0.485	0.242	0.667	0.666
40	+CO2	=N	1	16.5	0.06	0	0	0.493	0.507	0.553	0.907	0.907
41	+CO2	+N	1	8.75	0.05	0	0.006	0.49	0.503	0.699	0.884	0.885
42	+CO2	=N	1	0	1.75	1.75	0.545	0.227	0.227	0.944	0.953	0.953
43	=CO2	+N	16	0	2.06	2.03	0.581	0.208	0.211	0.923	0.934	0.934
44	+CO2	+N	1	9.97	0.01	0	0.003	0.497	0.5	0.949	0.978	0.978
45	+CO2	+N	1	10.86	0.22	0	0.002	0.471	0.527	0.81	0.934	0.935
46	=CO2	+N	1	9.08	0.21	0	0.006	0.471	0.524	0.835	0.937	0.938
47	+CO2	+N	1	16.05	0.59	0	0	0.426	0.574	0.886	0.953	0.955
48	=CO2	=N	1	0	3.26	3.25	0.718	0.141	0.141	0.909	0.916	0.916
49	+CO2	+N	1	6.09	0.05	0	0.023	0.482	0.494	0.748	0.87	0.871
50	=CO2	+N	1	5.89	0.51	0	0.029	0.424	0.547	0.662	0.839	0.845
51	+CO2	+N	1	10.94	0.08	0	0.002	0.489	0.509	0.879	0.932	0.932
52	=CO2	=N	1	7.62	0.04	0	0.011	0.49	0.499	0.651	0.851	0.851
53	+CO2	=N	16	1.72	0.17	0	0.181	0.391	0.427	0.856	0.904	0.905
54	+CO2	+N	1	11.35	0.02	0	0.002	0.497	0.501	0.312	0.793	0.793
55	+CO2	=N	16	11.61	0.85	0	0.002	0.395	0.603	0.828	0.943	0.946

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No	CO <sub>2</sub>	N	#Spp	1	2	3	1	2	3	1	2	3
56	+CO2	+N	1	18.4	0.35	0	0	0.456	0.544	0.717	0.946	0.948
57	+CO2	=N	16	10.4	0.4	0	0.003	0.449	0.548	0.93	0.959	0.961
58	=CO2	+N	1	3.45	0.1	0	0.084	0.447	0.469	0.621	0.787	0.788
59	=CO2	=N	16	2.62	0.21	0	0.124	0.415	0.461	0.877	0.916	0.917
60	+CO2	+N	16	7.28	0.31	0	0.014	0.455	0.531	0.898	0.951	0.953
61	=CO2	+N	16	6.81	0.15	0	0.017	0.473	0.51	0.362	0.726	0.729
62	=CO2	=N	1	3.34	0.25	0	0.091	0.426	0.483	0.779	0.872	0.874
63	+CO2	=N	16	0	1.1	0.97	0.456	0.263	0.281	0.859	0.889	0.89
64	+CO2	=N	16	2.31	0.6	0	0.153	0.36	0.487	0.677	0.797	0.807
65	+CO2	+N	16	15.57	0.55	0	0	0.431	0.569	0.804	0.952	0.954
66	+CO2	=N	1	7.77	0.47	0	0.011	0.437	0.552	0.801	0.915	0.918
67	=CO2	=N	1	6.61	0.17	0	0.019	0.47	0.511	0.77	0.895	0.896
68	+CO2	+N	1	11.54	0.11	0	0.002	0.485	0.513	0.85	0.951	0.952
69	=CO2	=N	16	0	4.13	4.1	0.797	0.101	0.103	0.813	0.816	0.816
70	=CO2	+N	16	9.54	0.34	0	0.005	0.456	0.539	0.829	0.932	0.934
71	=CO2	+N	1	6.36	0.21	0	0.021	0.464	0.514	0.872	0.939	0.94
72	=CO2	+N	1	15.78	0.59	0	0	0.426	0.574	0.61	0.912	0.916
73	=CO2	=N	1	12.16	0.4	0	0.001	0.449	0.55	0.754	0.925	0.927
74	=CO2	=N	16	0.64	0.02	0	0.267	0.365	0.368	0.861	0.902	0.902
75	+CO2	=N	1	4.92	0.14	0	0.042	0.461	0.496	0.909	0.951	0.952
76	=CO2	+N	16	0	4.16	4.16	0.8	0.1	0.1	0.939	0.94	0.94
77	=CO2	+N	16	6.67	0.38	0	0.019	0.444	0.537	0.908	0.95	0.951
78	+CO2	=N	1	3.76	0.14	0	0.073	0.447	0.48	0.885	0.933	0.933
79	+CO2	=N	16	4.92	0.48	0	0.046	0.42	0.535	0.87	0.932	0.934
80	+CO2	+N	16	0	3.03	2.31	0.651	0.143	0.205	0.547	0.593	0.617
81	+CO2	=N	1	2.34	0.13	0	0.138	0.417	0.445	0.716	0.826	0.828
82	+CO2	=N	16	8.33	0.26	0	0.008	0.463	0.529	0.849	0.939	0.94
83	+CO2	+N	16	6.49	0.87	0	0.023	0.384	0.593	0.874	0.924	0.93
84	=CO2	=N	1	11.48	0.28	0	0.002	0.464	0.534	0.739	0.915	0.917
85	+CO2	=N	1	12.28	0.06	0	0.001	0.491	0.507	0.927	0.962	0.962
86	+CO2	+N	16	1.07	0.48	0	0.247	0.331	0.422	0.67	0.774	0.783
87	+CO2	+N	1	2.31	0.54	0	0.152	0.367	0.481	0.809	0.879	0.885

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No	CO <sub>2</sub>	N	#Spp	1	2	3	1	2	3	1	2	3
88	+CO2	=N	16	2.37	0.1	0	0.135	0.421	0.443	0.733	0.837	0.838
89	+CO2	+N	16	0	1.99	1.99	0.575	0.212	0.213	0.956	0.961	0.961
90	+CO2	+N	16	7.55	0.75	0	0.013	0.402	0.585	0.799	0.907	0.913
91	=CO2	=N	1	0	1.49	1.32	0.502	0.239	0.26	0.815	0.85	0.852
92	=CO2	=N	16	7.77	0.25	0	0.011	0.463	0.526	0.64	0.854	0.857
93	=CO2	+N	1	11.41	0.18	0	0.002	0.477	0.521	0.652	0.892	0.893
94	=CO2	+N	1	5.98	0.1	0	0.025	0.476	0.499	0.888	0.946	0.946
95	=CO2	=N	1	2.5	0.42	0	0.136	0.387	0.477	0.58	0.744	0.753
96	=CO2	=N	16	5.67	0.65	0	0.033	0.405	0.562	0.886	0.942	0.945
97	=CO2	=N	1	5.38	0.17	0	0.034	0.463	0.503	0.737	0.871	0.873
98	+CO2	=N	1	23.04	0.67	0	0	0.417	0.583	0.717	0.962	0.964
99	=CO2	=N	1	1.74	0.11	0	0.177	0.4	0.423	0.902	0.934	0.934
100	=CO2	+N	16	6.96	0.41	0	0.017	0.441	0.542	0.866	0.939	0.941
101	+CO2	+N	1	0	4.29	4.29	0.811	0.095	0.095	0.94	0.94	0.94
102	=CO2	+N	16	12.27	0.12	0	0.001	0.485	0.514	0.837	0.947	0.948
103	=CO2	+N	1	14.19	0.21	0	0	0.474	0.526	0.631	0.909	0.91
104	=CO2	=N	1	2.94	0.14	0	0.106	0.431	0.463	0.744	0.85	0.851
105	=CO2	+N	1	6.44	0	0.06	0.02	0.497	0.483	0.095	0.604	0.603
106	=CO2	+N	1	1.55	0.18	0	0.194	0.385	0.421	0.837	0.893	0.895
107	=CO2	+N	1	5.77	0.19	0	0.028	0.463	0.509	0.558	0.792	0.795
108	=CO2	+N	16	0	1.89	1.77	0.555	0.216	0.229	0.784	0.819	0.821
109	=CO2	=N	1	2.54	0.51	0	0.136	0.377	0.487	0.863	0.913	0.917
110	=CO2	=N	1	5.21	0	0.03	0.036	0.486	0.478	0.369	0.693	0.693
111	=CO2	+N	16	2.09	0.07	0	0.152	0.416	0.432	0.903	0.937	0.938
112	+CO2	+N	1	12.4	0.18	0	0.001	0.477	0.522	0.645	0.897	0.898

**Table A.3** Regression parameters for linear regressions between the parameters for the two and three pool models

	Intercept	Slope	$r^2$
$k_f$	0.0006	1.028	0.997
$C_f$	0.0036	0.986	0.997
$k_s$	0.0000	1.754	0.935

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## Appendix B

# Below-ground biomass ANCOVA

**Table B.1** ANCOVA results for three pool model with below-ground biomass covariate

		$N$	$C_f$	$k_f$	$C_s$	$k_s$	$C_{NHC}$	$Total\ C$
CO <sub>2</sub>	ambient	56	0.09	0.033	3.78	0.00025	0.19	5.88
	elevated	56	0.084	0.043	3.96	0.00028	0.17	5.84
N	ambient	56	0.094	0.034	3.75	0.00029	0.18	5.67
	elevated	56	0.081	0.041	4	0.00025	0.19	6.06
Species number	1	64	0.085	0.042	3.78	0.00031†	0.18	5.82
	16	48	0.089	0.034	3.96	0.00023†	0.18	5.9
Root biomass		112	0.0017*	-0.00052	-0.000083	0.0010**	0.00011	0.000017
$R^2$			0.33	0.18	0.23	0.25	0.15	0.21

Mixed effects model parameter estimates of fast and slow carbon pool sizes ( $C_f$ ,  $C_s$ ,  $C_{NHC}$  and  $Total\ C$ : mg-C g-soil<sup>-1</sup>), and decomposition rates ( $k_f$  and  $k_s$ : day<sup>-1</sup>). † $P \leq 0.1$ , \* $P \leq 0.05$ , \*\* $P \leq 0.01$  (ANCOVA). No significant interactions were found  $P \leq 0.1$ .