Quantum Dots: A New Technique to Assess Mycorrhizal Contributions to Plant Nitrogen Across a Fire-Altered Landscape

Kathleen Treseder¹,²*, Matthew Whiteside¹, and David LeBauer²

Introduction

The overarching objective of our research is to develop a better understanding of the contributions of mycorrhizal fungi (root symbionts) to the nitrogen (N) nutrition of plants in habitat fragments in Southern California. We focused on habitat fragmentation because it is one of the most striking aspects of global change operating in Southern California. About 90% of coastal sage scrub habitat, for instance, has been lost due to urban development and agriculture. Nitrogen uptake is an important issue in restoration and conservation of remaining fragments, because the growth of many plant species is N limited.

For Objective 1, we developed a new nanotechnological technique—quantum dots—that allowed us to quantify rates of uptake of organic N into mycorrhizal fungi and their host plants. We found that arbuscular mycorrhizal fungi (AMF) were capable of assimilating organic N derived from recalcitrant molecules, which is contrary to prevailing ideas about the role of AMF in nutrient cycling (Whiteside et al. 2009).

For Objective 2, we examined consequences of habitat fragmentation in coastal sage scrub on the abundance of AMF and ectomycorrhizal (ECM) fungi, and on the activities of extracellular enzymes targeting organic compounds in the soil. We found that AMF dominated the mycorrhizal community more in smaller fragments than in larger fragments, potentially because AMF are more tolerant of disturbance than are ECM fungi. In addition, as fragment size decreased, so did activities of five extracellular enzymes that break down organic material in the soil.

For Objective 3, we extended our assessment to a very wide gradient of habitat size, ranging from 23 to 8.9 x 10⁹ m². The gradient comprised 14 natural ecosystems in Southern California, including the coastal sage habitat fragments mentioned above. We used 18S rDNA sequencing to characterize the community composition of fungi in these sites. We observed that fungal community composition varied with habitat size, suggesting that large-scale habitat fragmentation could influence even these small-scale organisms. Moreover, fungal communities that were located far from one another tended to be less similar compared to neighboring communities; this relationship was particularly evident at distances up to 200 km. It is possible that distance between habitats could have influenced dispersal of fungal communities from one habitat to another, although spatial autocorrelation of environmental characteristics could also have contributed to the pattern.

For Objective 4, we conducted a data synthesis to determine the prevalence of N limitation of net primary productivity (NPP). We found that N limitation was widespread globally, and was evident in every biome except deserts. These results indicate that the contribution of mycorrhizal

*Principal Investigator
For more information contact Dr. Kathleen Treseder (Treseder@uci.edu).
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fungi to the breakdown and uptake of organic N may have consequences for carbon inputs into ecosystems over a broad distribution of ecosystems.

These results are relevant to the Kearney Mission, because they address the influx of carbon into ecosystems (via nutrient uptake by plants through mycorrhizal fungi) as well as efflux of carbon dioxide from ecosystems (via decomposition by saprotrophic fungi). Both processes are important ecosystem services relevant to society. We examined scale-dependent fungal dynamics as a function of habitat fragmentation, over spatial scales ranging from 23 to $8.9 \times 10^9$ m$^2$ and from individual ecosystems to the region of Southern California. Results are relevant to management issues, because they address habitat fragmentation, a major element of environmental change in California. In addition, we have partnered with the Orange County Society for Conservation Biology, who is restoring the coastal sage scrub habitat surrounding our smaller fragments (http://www.ocscb.org/restoration.shtml). We provide ecological advice regarding soil management and plantings, based on our experimental findings.

Objective 1: Development of a nanotechnological technique to measure the uptake of organic N by fungi and plants.

Objectives

One recent subject of debate is the extent to which plants are able to directly consume organic nitrogen (ON). Traditionally, ON was thought to undergo a transformation into inorganic N before being taken up by plants (Liebig 1843). This theory assumes N mineralization is the limiting process for plant growth in most ecosystems (Vitousek 1997). However, isotope labeling has provided evidence that plants can take up ON, at least as simple forms such as amino acids (Turnbull et al. 1995, Lipson and Monson 1998, Näsholm et al. 1998, Lipson and Näsholm 2001, Miller and Cramer 2005, Rains and Bledsoe 2007). It is unclear, however, whether ON molecules are directly assimilated by plant roots or if plants acquire ON indirectly via mycorrhizal fungi or other microbial symbionts.

Arbuscular mycorrhizal fungi are common worldwide, and form symbioses with approximately 80% of plant families (Newman and Reddell 1987, Smith and Read 1997). They mine the soil for nutrients and translocate a portion to their host plants in exchange for carbon (Smith and Read 1997). The prevailing paradigm is that AMF specialize in the capture of inorganic nutrients such as phosphate, ammonium, and nitrate (Read 1991, Smith and Smith 1997). Uptake of ON by AMF has been challenging to investigate in natural systems because of technical difficulties in tracing the flow of organically-derived nutrients from the soil into hyphae.

One promising technique to investigate the flow of nutrients between plants, microorganisms, and soil in field conditions is the use of quantum dots. Quantum dots (QDs) are nanoscale semiconductors that fluoresce in different colors depending on their size (Chan and Nie 1998, Wong and Stucky 2001, Reiss et al. 2002, Alivisatos et al. 2005). They are typically composed of a cadmium selenide core wrapped in a zinc sulfide shell, and they can be enclosed by protective polymer coatings (Fig. 1)(Dubertret et al. 2002). Polymer coated QDs are commercially available, and range in diameter from 2 nm (NNT, Ontario, Canada) up to 20 nm (Invitrogen, Carlsbad, CA). There are many advantages of QDs over traditional labels and tags.
Quantum dots have broad absorption spectra with very narrow emission peaks, meaning multiple colors can be assessed on the same sample by using the same light source. Since QDs are resistant to metabolic and chemical degradation and are not susceptible to photobleaching, they are effective tracers for long term studies (Alivisatos et al. 2005, Michalet et al. 2005). In addition, QDs can be bound to amino or carboxyl groups within organic compounds. In this way, the movement of these compounds through soils or organisms can be imaged and tracked (Dubertret et al. 2002, Kloepfer et al. 2005). To our knowledge, this study was the first to apply quantum dot technology to ecological questions.

We assessed QDs as a tool to observe the translocation of ON in laboratory and soil-based systems. Specifically, our objectives were to use QDs to track ON uptake (labile and recalcitrant) by a model group of non-mycorrhizal fungi, *Penicillium solitum*, and by AMF-colonized plants. By testing this technique on *Penicillium* fungi, we assessed uptake of QD-substrates in a known ON-acquiring saprotroph as a proof-of-method. We then extended this method to AMF to examine ON uptake by this group. We used glycine and arginine (common amino acids) to represent labile ON, and chitosan (deacylated form of chitin) to represent recalcitrant ON.

**Approach**

We conjugated quantum dots to the amino groups of glycine, arginine, and chitosan, and incubated them with *Penicillium* fungi (a saprotroph), and annual bluegrass (*Poa annua*) inoculated with AMF. As experimental controls, we incubated fungi and bluegrass samples with substrate-free quantum dots as well as unbound quantum dot substrate mixtures. We also “field-tested” quantum dots by applying them to soils. In this case, minirhizotron cameras were used to image the movement of quantum dots belowground, and UV flashlights were used to visualize quantum dot uptake in plant shoots.

**Results**

*Penicillium* fungi, annual bluegrass, and AMF all showed uptake and translocation of quantum dot-labeled organic N, but no uptake of quantum dot controls (Fig. 1). Quantum dots in soil-based systems could be tracked belowground in minirhizotron images, and could be viewed aboveground in plant shoots with the naked eye (Fig. 2).

**Discussion:**

Our results suggest that the QD-labeled ON is taken up and transported to particular structures in fungi and plants, depending on the type of ON compound conjugated to the QD. In natural settings, proteinaceous materials (e.g., amino acids, peptides, and proteins) comprise 40% of soil nitrogen and amino sugars comprise 5-6% (Schulten and Schnitzer 1998). As reviewed by Rentsch et al. (2007) and documented by Paungfoo-Lonhienne et al. (2008), saprotrophic and ectomycorrhizal fungi are known to directly acquire amino acids, peptide chains and proteins and plants contain transporters for amino acids, peptides and proteins. Additionally, transportation of chitin and other polysaccharides is not well studied. Previous studies have shown that *Penicillium* fungi take up and compartmentalize intact amino acids such as glycine and arginine (Kitamoto et al. 1988, Hillenga et al. 1996) and are capable of breaking down and absorbing more recalcitrant forms of ON such as the polysaccharide chitin (Binod et al. 2007). *Penicillium* fungi compartmentalize nutrients and amino acids in two main locations: in vacuoles where they can be stored, and in cytoplasm where they can be quickly metabolized or transformed (Griffin...
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1994). Vacuoles of *Penicillium* hyphae can contain up to 51% of total cellular glycine and up to 98% of total cellular arginine (Kitamoto et al. 1988, Roos et al. 1997). We observed QD-chitosan in cytoplasm and QD-glycine in vacuoles, but QD-arginine was observed in cytoplasm and not vacuoles. The lack of QD-arginine in vacuoles is most likely the result of QD-arginine size after conjugation. Glycine contains one amino terminal as a potential QD binding site, however, arginine contains two. Therefore, it is possible for each arginine molecule to bind with more than one QD during conjugation. QD-arginine (theoretically twice as large as QD-glycine) may be small enough to pass through the cell wall, but too large to pass into the vacuole.

Contrary to the prevailing paradigm, AMF took up labile (QD-glycine) and recalcitrant (QD-chitosan) forms of ON. QD-chitosan was located within the cytoplasm after 5 h of incubation, similar to *Penicillium solitum*. QD-glycine was located in tube shaped vacuoles, complimenting AMF location of phosphate in previous studies (Ashford 2002, Uetake et al. 2002). The red QDs used were encased in a polymer with carboxyl terminals, so even if the ON attached was mineralized it was still bound to a carboxyl group of the QD polymer. Since QDs were taken up only when bound to ON compounds, it appears that ON was acquired directly and actively by AMF.

A few studies have demonstrated that AMF can obtain N derived from organic sources (Clique et al. 1997, Hawkins et al. 2000, Hodge et al. 2001, Rains and Bledsoe 2007), but without direct imaging it is difficult to determine if AMF can take up N in organic form. In a laboratory microcosm, Hodge et al. (2001) placed 15N-labeled litter in soil compartments that could be accessed by AM hyphae but not by plant roots. In doing so, they demonstrated that AMF can acquire N from organic material. However, this approach cannot be easily replicated under field conditions. Mesh-enclosed cores could be installed that allow ingrowth by fungal hyphae but not roots. Unfortunately, though, it is difficult to prevent ON from diffusing to or from the core. Hobbie & Hobbie (2006) recently used natural abundance of 15N in plants and fungi to estimate that ectomycorrhizal fungi are responsible for 61–86% of N uptake by plants in arctic tundra. However, these calculations require that all pools of available N in the soil display similar 15N signatures. This condition may not be met in many ecosystems.

We determined that QDs may be used in conjunction with field imaging techniques. Quantum dots of any color will fluoresce under standard UV light. Therefore, QDs are commonly imaged using epifluorescent and confocal microscopy (Voura et al. 2004, Chen 2006). These methods incorporate continuous viewing coupled with light intensity detectors or software, providing quantitative results comparable to fluorescent spectrophotometers (Yezhelyev et al. 2007). Mycorrhizae and plant roots can be imaged in a similar manner using underground minirhizotron cameras. Minirhizotron cameras are commonly fitted with an UV light source and can take photos of 4 μm resolution (Hendrick and Pregitzer 1996). We did not quantitatively examine QD uptake in this study. However, we did use minirhizotron imaging to confirm ON uptake by fungal hyphae in soil. Moreover, we found that QDs can be used with portable handheld UV lights to observe uptake in grass shoots with the naked eye. This method would allow a researcher to apply QD-labeled nutrients to natural systems and non-destructively determine uptake among plants in real-time.

Our study contributes to the growing body of literature demonstrating that AMF may have a more significant effect on ON dynamics than previously believed (Näsholm et al. 1998, Hawkins
et al. 2000, Hodge et al. 2001, Rains and Bledsoe 2007). The QD technique provides a simple, inexpensive, and non-destructive measure of mycorrhizal fungi and root uptake in natural systems. By using this technique to trace the uptake of ON molecules, researchers could improve our knowledge of nutrient acquisition by organisms in general, and the role of AMF in plant ON uptake in particular.

This work has been published in Ecology (Whiteside et al. 2009), and excerpts from the publication have been included above.

Objective 2: Mycorrhizal abundance and extracellular enzyme activity in a size gradient of fragments of coastal sage scrub.

Objective

Habitat fragmentation is frequently linked to loss of habitat quality. Most models assume that fragment isolation and fragment size are the main drivers of habitat quality via island biogeography mechanisms (MacArthur and Wilson 1967, Hanski and Simberloff 1997). However, empirical studies suggest edge effects related to exposure to humidity, light, wind, and physical disturbance have greater impacts (Soule et al. 1992, Harrison and Bruna 1999). For example, wind can damage trees at the edges of a fragment (Malcolm 1994), resulting in a decrease in plant biomass. Thus, based on perimeter to area ratios, we would expect edge effects to be negatively correlated to fragment size, where smaller fragments suffer more disturbance per unit area.

We hypothesized that habitat fragmentation alters mycorrhizal communities through two potential mechanisms. First, habitat fragmentation may exclude plant species that harbor ECM fungi if ECM host plants (e.g., certain shrubs and trees) require greater undisturbed area to establish. Second, ECM fungi may be more sensitive to direct disturbance than are AMF.

Ectomycorrhizal fungi are thought to possess a greater uptake capacity for organic N than do AMF (Read 1991, Read and Perez-Monreno 2003). If AMF target organic N to a lesser extent than do ECM fungi, a shift in abundance ratio between ECM and AM fungi under fragmentation may occur. As a result, ecosystem-level exploitation of organic N by plants should decline, followed by a reduction in standing plant biomass and NPP.

Approach

We selected six habitat fragments with areas that ranged from 23 to 5414 m². These fragments are located in Newport Back Bay, Orange County. They are surrounded locally by foot and horse trails and regionally by urban development. We collected data on mycorrhizal community structure, plant canopy height, plant diversity, and activities of five extracellular enzymes produced by microbes to break down organic N (N-acetyl-glucosaminidase, glycine aminopeptidase, and leucine aminopeptidase) and organic carbon (β-glucosidase and cellobiohydrolase).

Results

We found that plant diversity declined with decreasing habitat area (Fig. 3). This decline is accompanied by a reduction in maximum canopy height in the smaller fragments. Likewise,
ECM fungi tend to represent a smaller portion of the mycorrhizal community as fragment size diminishes, although this pattern is only marginally significant.

Enzyme activity in organic soil (Oe-horizons) was significantly positively correlated with fragment size for five extracellular enzymes: N-acetyl-glucosaminidase (P = 0.004), β-glucosidase (P = 0.035), glycine aminopeptidase (P = 0.022), leucine aminopeptidase (P = 0.019), and cellobiohydrolase (P = 0.022, Fig. 4).

Discussion

Our results to date support our predictions that a reduction in habitat size has consequences for plant diversity, mycorrhizal community structure, standing plant biomass, and nutrient cycling. The shift in mycorrhizal fungi may occur because ECM host plants are less abundant in the smaller fragments. Other studies have found that ECM fungi can decline substantially after a disturbance (e.g., trampling and grazing), and can require decades to recover (Visser 1995, Dahlberg et al. 1997, Grogan et al. 2000). In contrast, AMF are more resilient (Allen et al. 1998, Korb et al. 2003) and often need only a few years to return to pre-disturbance levels (Korb et al. 2003).

The decline in extracellular enzyme activity in smaller fragments could have resulted from the decrease in relative ECM abundance, if ECM fungi contribute disproportionately to production of these enzymes. Although our quantum dot study indicated that AMF can access recalcitrant organic N, direct comparisons between AMF and ECM fungi in organic N breakdown have yet to be performed.

Objective 3: Biogeography of soil fungi across a wide gradient in habitat size

Objective

We examined fungal community composition across a broad gradient in habitat size in Southern California. Soil fungi control processes that are important in ecosystem function and of interest to land managers. As fungi decompose litter, they release greenhouse gases to the atmosphere, and they transform nutrients to forms used by plants and other organisms. Moreover, the community composition of fungi can influence decomposition rates, as indicated by microcosm studies in the lab (LeBauer, 2009; Setala and McLean, 2004). In addition, taxa may vary in their responses to environmental parameters such as plant community composition and disturbance, which could determine the degree to which fungal communities are sensitive or resilient to changes in the environment. Altogether, these responses could influence carbon dynamics in soils of Southern California.

Approach

We determined fungal biodiversity in soil collected from 14 sites in Southern California, including our coastal sage scrub fragments, as well as grasslands, and deserts. Soils from the grasslands and deserts are the same sampled used in an analysis of global bacterial diversity conducted by Fierer and Jackson (2006). Samples were collected near the peak of the growing season in each site. We sequenced the 18s rDNA region of 68 to 1540 DNA strands from each
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site. Non-metric scaling (NMS) was used to transform community composition to three dimensions based on relative abundance of individual taxa.

Results

We found that fungal community composition was significantly related to habitat size (along Dimension 1) and ecosystem type (Dimension 2) across Southern California (Fig. 5). In addition, neighboring fungal communities were more similar to one another than were remote communities, and this pattern was especially evident at distances less than 200 km (Fig. 6).

Discussion

The relationship between fungal community composition and habitat size was surprising, given that fungi are microscopic and are thought to be easily dispersed. One might expect that fungi are broadly and easily distributed among ecosystems, and that they would be affected more by environmental characteristics of the microsites that they inhabit rather than disturbance some distance away. However, changes in plant community composition with habitat size may have indirectly affected fungal communities by altering the chemistry and abundance of available substrates. The differences in fungal community composition among ecosystem types may also have resulted from differences in plant community composition.

The relationship between community similarity and distance also indicates that fungi may be dispersal limited, even among ecosystems that are within 200 km of one another. An alternate explanation for this pattern could be spatial autocorrelation of environmental factors—sites that are closer to one another may share similar environmental characteristics. The mobility of microbes is a matter of debate (Martiny et al., 2006). Baas Becking (1934) suggested that microbial taxa are easily dispersed, stating “Everything is everywhere, but the environment selects.” Indeed, recent analyses in central Europe have revealed that fungal diversity in air samples is equivalent to that found in soils (Frohlich-Nowoisky et al., 2009). Fungi of the phylum Basidiomycota were better represented in the air samples than were those of Ascomycota and Fungi Incertae Sedis, possibly owing to the basidiomycetes’ relative efficiency of spore dispersal (i.e., dry release from aboveground fruiting bodies) and more aerodynamic spores (Levetin, 1990).

Objective 4: A global synthesis of plant responses to N additions.

Objectives

Nitrogen (N) constrains net primary production (NPP) in terrestrial ecosystems (Vitousek and Howarth 1991). Nitrogen limitation is diagnosed when addition of N results in increased NPP. In this manner, a broad array of ecosystem-scale studies has consistently demonstrated N limitation (Kenk and Fischer 1988, Tamm 1991, Hooper and Johnson 1999). The persistence of N limitation results from the transient nature of biologically available forms of N (Vitousek et al. 2002). Unlike the abiotic inputs of many other resources, the primary source of N is biological N fixation. Once fixed, N is particularly susceptible to being removed from an ecosystem by leaching and volatilization.
Anthropogenic N fertilization of N-limited ecosystems is a primary component of global change. During the 20th century, anthropogenic N fixation doubled the global flux of N to the biosphere. Moreover, N deposition rates are expected to increase another two or three-fold before plateauing (Vitousek et al. 1997, Galloway and Cowling 2002, Lamarque et al. 2005). In contrast to the current pattern of N deposition, which is concentrated in temperate regions of the northeastern US and northern Europe, future N deposition will increasingly occur in the tropical regions (Galloway and Cowling 2002, Lamarque et al. 2005). This change in distribution of N deposition will result in N fertilization of a larger proportion and diversity of the earth’s ecosystems. However, the effect of this fertilization on global NPP will depend on the degree to which N limits NPP in both temperate and tropical ecosystems.

To assess global patterns of N limitation, we conducted a meta-analysis of N limitation across diverse biomes. Following the geophysical model (Walker and Syers 1976), we predicted that N limitation would increase poleward, and with temperature or precipitation limitation of N mineralization (Vitousek and Howarth 1991, Reich and Oleksyn 2004).

**Approach**

To test our predictions, we performed a comprehensive meta-analysis on N addition studies, using the response ratio R as an estimate of the ratio of production in fertilized to control plots. We found 80 publications containing 126 independent N addition studies that measured the response of plant productivity to N.

**Results**

We found that most ecosystems are N limited with an average 29% growth response to N (i.e., R=1.29). R was significant within temperate forests (R=1.19), tropical forest (R=1.60), temperate grasslands (R=1.53), tropical grasslands (R=1.26), wetlands (R=1.16), and tundra (R=1.35), but not deserts. Eight tropical forest studies had been conducted on very young volcanic soils in Hawai‘i, and this subgroup was strongly N-limited (R=2.13). The degree of N-limitation in the remainder of the tropical forest studies (R=1.20) was comparable to that of temperate forests. Moreover, forest R did not vary with latitude. Grassland response increased with latitude, but was independent of temperature and precipitation.

**Discussion**

Nitrogen limits ANPP in the majority of terrestrial ecosystems. Our results demonstrate consistent and statistically significant responses of ecosystem NPP to N addition (Table 1), supporting the hypothesis that N limitation is widespread (Vitousek and Howarth 1991). We focus on N limitation because increasing N availability is a major component of global change (Vitousek and Howarth 1991). However, other nutrients stimulate NPP, and responses to N in combination with other nutrients exceed responses to N alone (Harpole et al. 2007, Elser et al. in press). Therefore, our estimates of R should be considered conservative with respect to nutrient limitation overall.

Although grassland R increased with latitude, there was no correlation between R and MAT or MAP. These results support the conclusion that grassland R is independent of climate over wide ranges (Schimel et al. 1997, Hooper and Johnson 1999). Hooper and Johnson (1999) analyzed the results of forty arid to semi-arid (MAP = 211-1031mm/yr) grassland N-addition
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studies, including multiple time points and fertilization rates from each study, and observed no correlation between R and geographical or temporal variation in precipitation. Eight of our thirty-eight grassland data points were included in the previous study.

The CENTURY model (Schimel et al. 1997) predicts tight coupling of water and N limitation. This occurs because both soil N availability and plant N demand are limited by, and therefore positively correlated to, temperature and water availability. According to CENTURY (Schimel et al. 1997), this causes the ratio of N availability to plant N demand to remain constant as temperature and precipitation increase. By corollary, the ratio of N deficit (demand – supply) to N demand would also remain constant. If plant response to N is proportional to the N deficit while plant N demand is proportional to NPP, R should also remain constant as temperature and precipitation increase. Our finding that grassland R remains constant across a large range of MAT and MAP supports the CENTURY prediction (Schimel et al. 1997) and corroborates the results reported by Hooper and Johnson (1999).

The response of plants to N addition is an indication of the global NPP deficit that can be attributable to N limitation. However, we do not expect that long term N-deposition will produce the same NPP response that was observed in the N fertilization studies. Experimental nitrogen addition provides an ecosystem with an immediate and large increase in N availability. In the present analysis, the average rate of N addition was 14.3 (± 1.1 SE) g N m\(^{-2}\) y\(^{-1}\). In contrast, anthropogenic pollution produces a steadily increasing rate of N deposition from pre-industrial levels. Presently, anthropogenic N inputs average 1g N m\(^{-2}\) y\(^{-1}\) over terrestrial ecosystems, and could level off at 4-6 g N m\(^{-2}\) y\(^{-1}\) during the next century (Prather et al. 2001, Galloway and Cowling 2002, Lamarque et al. 2005). On average, we found a 30% increase in NPP under N addition, and at the global scale this is equivalent to an NPP response of 20 x 10\(^{15}\) g C y\(^{-1}\) (Calculated using R from Table 1 and global NPP estimates for each biome, (Houghton and Skole 1990, Saugier et al. 2001)). However, our data set represents short-term data that are not necessarily applicable to persistent low level N deposition. Global models estimate that the present impact of anthropogenic N fertilization on global NPP is closer to 0.5-1.5 x 10\(^{15}\) g C y\(^{-1}\) (Townsend et al. 1996, Vitousek et al. 1997). Furthermore, there is evidence that long term exposure to pollution can negatively impact plant growth as a system becomes N saturated and the soil becomes acidified (Aber et al. 1998).

Ultimately, the balance between production and decomposition determines the impact of N on the net global flux of carbon from the biosphere to the atmosphere. Anthropogenic N can augment terrestrial carbon storage by increasing NPP more than decomposition (Vitousek et al. 1997). Our study supports this proposition by demonstrating strong, consistently positive effects of N on NPP in contrast to the inconsistent effects of N on decomposition (Fog 1988). Nitrogen can increase, decrease or have no effect on the rate of decomposition, depending on environmental conditions, substrate quality, and microbial physiology (Schlesinger and Andrews 2000). For example, a meta-analysis of litter decomposition experiments by Knorr et al (2005) found no significant effect of N on litter decomposition overall. In regions exposed to high rates of N deposition, experimental N addition either decreased or had no effect on decomposition rates (Knorr et al. 2005). Conversely, N limits NPP even among ecosystems in our meta-analysis that were exposed to high background rates of N deposition.
N limitation constrains productivity in most ecosystems, and the degree of limitation varies by biome. Response was correlated with latitude, MAT, or MAP within some biomes but not overall. Our findings support the prediction that N limitation is widespread (Vitousek and Howarth 1991), but do not rule out multiple element limitation (Rastetter and Shaver 1992, Elser et al. in press). Evidence for the poleward increase in the degree of N limitation as predicted by the geophysical hypothesis (Walker and Syers 1976) was observed in grasslands, but not forests. Nitrogen limitation appears to be important in most tropical forests. Additionally, temperate forest R may be suppressed by historically elevated N deposition. Increasing N deposition, particularly in the most rapidly developing regions, is likely to further stimulate global NPP and slow the accumulation of atmospheric CO₂.

This work has been published in *Ecology* (LeBauer and Treseder 2008), and excerpts from that publication are included above.
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Figures

Figure 1. Superimposed (white light and fluorescence) confocal laser scans of *Penicillium solitum* uptake of QD-ON conjugates. (A) At 2 hrs of incubation with QD-glycine, fungal hyphae showed evidence of uptake. (B) At 6 hrs, less labeled glycine was seen in solution and more within the hyphae. (C) After 24 hrs very little labeled glycine was seen outside of the hyphae. (D) After 24 hrs QD-arginine fluorescence appeared in the cytoplasm but not in vesicles (arrows). (E) Uptake of QD labeled chitosan after 5 hrs of incubation. (F) QD control (quantum dots unbound to glycine) after 24 hrs of incubation showed no signs of uptake. Scale bar is 10 μm.
Figure 2. Fluorescence of QD-glycine in a plant and fungi using field imaging techniques. (A-D) Unmagnified digital images of a *Poa annua* individual incubated with orange excitation QD-glycine. (A) White light view (B) UV view (C) Root detail (D) Blade detail. Orange fluorescence indicates presence of QDs. Scale bar is (A, B) 1 cm, (C) 5 mm, (D) 500 µm. (E-G) Minirhizotron images of fungal hyphae uptake of QD-glycine (E) White light image pre-QD injection (F) UV image pre-QD injection (G) 2 hrs after injection of QD-glycine, UV minirhizotron images showed hyphal uptake (arrow) of the labeled glycine. Hypha is ~2mm long.
Figure 3. Plant diversity, canopy height, and mycorrhizal community structure in a size gradient of coastal sage scrub fragments in Newport Back Bay.
Figure 4. Extracellular enzyme activity in organic soil horizons (O<sub>1c</sub> horizons) was significantly correlated with fragment size for five enzymes. The best-fit lines are shown with corresponding $R^2$ values. The x-axis denotes fragment area ($m^2$).
Figure 5. Differences in fungal community composition in relation to habitat size (upper panel) and ecosystem type (lower panel). Each symbol represents one site. Ecosystem types did not differ significantly in habitat size along Dimension 1 ($P = 0.121$).
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Figure 6. Relationship between similarity of fungal communities and geographic distance among sites in Southern California. Each symbol represents a pairwise comparison between two sites.
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