Soil carbon sequestration in prairie grasslands increased by chronic nitrogen addition

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Abstract. Human-induced increases in nitrogen (N) deposition are common across many terrestrial ecosystems worldwide. Greater N availability not only reduces biological diversity, but also affects the biogeochemical coupling of carbon (C) and N cycles in soil ecosystems. Soils are the largest active terrestrial C pool and N deposition effects on soil C sequestration or release could have global importance. Here, we show that 27 years of chronic N additions to prairie grasslands increased C sequestration in mineral soils and that a potential mechanism responsible for this C accrual was an N-induced increase in root mass. Greater soil C sequestration followed a dramatic shift in plant community composition from native-species-rich C_4 grasslands to naturalized-species-rich C_3 grasslands, which, despite lower soil C gains per unit of N added, still acted as soil C sinks. Since both high plant diversity and elevated N deposition may increase soil C sequestration, but N deposition also decreases plant diversity, more research is needed to address the long-term implications for soil C storage of these two factors. Finally, because exotic C_3 grasses often come to dominate N-enriched grasslands, it is important to determine if such N-dependent soil C sequestration occurs across C_3 grasslands in other regions worldwide.

Key words: biodiversity; C_3 grasses; C_4 grasses; ecosystem functioning; ecosystem services; fire; nitrogen deposition; root mass; soil carbon sequestration.

Introduction

Human activities over the last century have greatly increased the terrestrial deposition of biologically available N while reducing terrestrial biological diversity (Vitousek et al. 1997, Stevens et al. 2004, Clark and Tilman 2008, Galloway et al. 2008). Greater availability of biologically active N can also influence the biogeochemical coupling of C and N cycles in soil ecosystems (Luo et al. 2004). N-induced effects on ecological processes include changes in (1) organic matter decomposition in litter and soils (Hobbie 2008, Keeler et al. 2009); (2) belowground C allocation including changes in root and mycorrhizal exudation (Giardina et al. 2004) and changes in C allocation to different soil C pools (Cusack et al. 2011); and (3) microbial composition and activity such as the suppression of heterotrophic respiration (Janssens et al. 2010, Lu et al. 2011) or the inhibition of extracellular enzyme activities (Ramirez et al. 2012). Thus increases in biologically active N may strongly affect the C sequestration ability of soils, which are the largest active terrestrial pool of organic C (Post et al. 1982). Although the mechanistic understanding of these ecological processes has greatly improved over the last decade, it remains unclear why N additions may have either positive, negative, or no effect on total soil C

pools (de Vries et al. 2006, Pregitzer et al. 2008, Janssens et al. 2010, Liu and Greaver 2010, Lu et al. 2011). Thus, the effects of chronic N fertilization on the total C content of mineral soils remains a key question, as are the underlying mechanisms that control such N-dependent changes in soil C stores.

Here, we address whether chronic N additions will affect the ability of soil mineral pools to act as long-term C sinks. Second, we address how chronic N additions contribute to changes in root mass and root C:N ratios. It is often thought that N fertilization decreases root competition for N and leads to lower C investment in roots (Liu and Greaver 2010). This however is not always the case and changes in root mass under increasing N additions were found positively related to changes in soil C storage (Van Groenigen et al. 2006, Lu et al. 2011). Third, we address how N-induced effects on soil C sequestration may be associated with shifts in plant species composition toward more "nitrophilous" plant communities (Wedin and Tilman 1996, Bobbink et al. 2010). This is a potentially important issue because various human activities often increase the abundance of exotic nitrophilous plant species (Bobbink et al. 2010, Ehrenfeld 2010). We address these potential chronic N addition effects by using 27 years of data from two experiments established in Minnesota, USA, where replicate plots in prairie grasslands have received very different rates of N addition since 1982. We applied N fertilization rates of 10 and 20 kg N·ha^{-1}·yr^{-1}, which

represent common rates of anthropogenic N deposition across many regions worldwide (Bobbink et al. 2010) as well as higher rates of N addition (>20 kg N·ha^{-1}·yr^{-1}) to address potential ecosystem responses to even greater N enrichment.

METHODS

We conducted our study at Cedar Creek Ecosystem Science Reserve, Minnesota, USA (45°24′ N, 93°12′ W). The soil is a well-drained fine sand, poor in N, with a low percentage of organic matter (2.38%; Clark et al. 2009). In 1982, an experimental grid was established in each of two later successional prairie-like grassland fields (i.e., fields B and C), which where abandoned from agriculture in 1957 and 1934, respectively. In field B, the experimental grid was disked before the establishment of 54 plots (16 m^2 each), which received six replicates of nine treatments randomly assigned within the grid. Treatments consisted of control (no nutrient addition), addition of non-N nutrients (P, K, Ca, Mg, S, and trace metals), and addition of non-N nutrients plus one of the seven levels of N addition (10, 20, 34, 54, 95, 170, and 270 kg N·ha^{-1}·yr^{-1}). N was added as pelletized NH_4NO_3 (34-0-0) twice each year, with half added in mid-May, and half in late June. Plots received annual wet nitrogen deposition of approximately 6 kg·ha^{-1}·yr^1 (58% NH_4, 42% NO_3). Mean N deposition was estimated using data in the EPA CASTNET program for total wet and dry inorganic nitrogen deposition (data *available online*).[4] We used average data for N deposition at the three nearest sites to Cedar Creek in the EPA CASTNET program (sites PRK134, STK138, and VOY413). This suggested that wet deposition is 72–84% of total N deposition, and is thus a reasonable proxy for total N deposition (see Clark and Tilman 2008). In the spring of 1992, three randomly chosen replicates of each nutrient treatment in field B were burned each spring. Field C also included 54 plots (16 m^2 each), established in 1982, which received the same six replicates of nine nutrient treatments randomly assigned within the grid as in Field B.

Soil sampling and analyses

Soil C and N samples were collected in mid-June 2009 to a soil depth of 20 cm (using a 3 cm diameter soil corer) for each of six sites per experimental plot in each of the 108 plots. Before collecting the mineral soil, plant litter, if present, was removed (generally being about 0 to at most 2 cm deep). We did this to allow comparison to previous soil samples that had been collected with the same methodology in 1982 for both fields B and C. Soil samples collected in 2009 from each plot were then sieved (through a 2 mm mesh size) to remove roots, mixed, and ground. Soil samples were then dried at 40°C for 5 days and stored in glass vials. Archived soil samples from 1982 and fresh soils from 2009 were all

[4] http://epa.gov/castnet/javaweb/mapcharts.html

analyzed for total C and N by combustion and gas chromatography (ECS 4010 gas chromatograph; COS-TECH Analytical, Valencia, California, USA). Ash samples analyzed after furnace burning (16 hours at 550°C) revealed negligible inorganic C. One soil sample per plot was collected between 2–20 cm soil depth using a PVC corer 8 cm in diameter to measure soil bulk density (i.e., mass of dried soil divided by soil volume).

Plant aboveground and belowground sampling

The 108 plots (field B and C) were sampled for above- and belowground biomass in June 2009. Aboveground plant biomass, which is a measure of aboveground productivity (NPP), was collected by clipping, drying, and weighing three parallel and evenly spaced 0.1 m × 3.0 m vegetation strips per plot. Plant litter was also collected within the same strips after the plant aboveground biomass harvest. Plots were sampled for belowground biomass by collecting three evenly spaced soil cores in each of the three clipped strips. Each core was 5 cm in diameter. Soil cores were washed with a gentle spray of water over a fine mesh screen until roots were free of soil. Roots were then dried; any soil residual was removed and roots were then weighed. Aboveground and belowground biomass was dried at 65°C until constant mass and then ground and analyzed for total C and N following standard methods on a 1500 NA Carlo-Erba element analyzer (CE Elantech, Lakewood, New Jersey, USA).

Potential net soil N mineralization rates

We performed laboratory incubations of soils collected from each of the 108 plots in June 2009 to assess net N mineralization rates. Soil samples were collected to 20 cm soil depth from three sites within each plot on 18 June 18 mixed, extracted with 1 mol/L KCl, shaken for 0.5 h, settled overnight at 4°C, and analyzed for NH_4^+-N and NO_3^--N with a Bran-Luebbe AA3 auto analyzer (Bran-Luebbe, Mequon, Wisconsin, USA). An additional 25-g subsample from each plot was incubated for 30 days in a dark room at 22°C after roots were sieved out. Sufficient water was added to each sample to keep moisture constant. After 30 days, soil samples were extracted and analyzed for NH_4^+-N and NO_3^--N as above. To determine net N mineralization final extractable concentrations of NH_4^+ and NO_3^- per gram of soil were subtracted from initial extractable concentrations per gram of soil.

Data analysis

To determine the potential effects of N additions on different ecosystem variables we performed nonlinear regression analyses (Appendix: Table A1). We found that the response of each ecosystem variable to the two non-N additions (control and the addition of non-N nutrients) was never statistically significant so we used the average of these two treatments in our analyses. For soil N mineralization rates, we log-transformed our data to meet
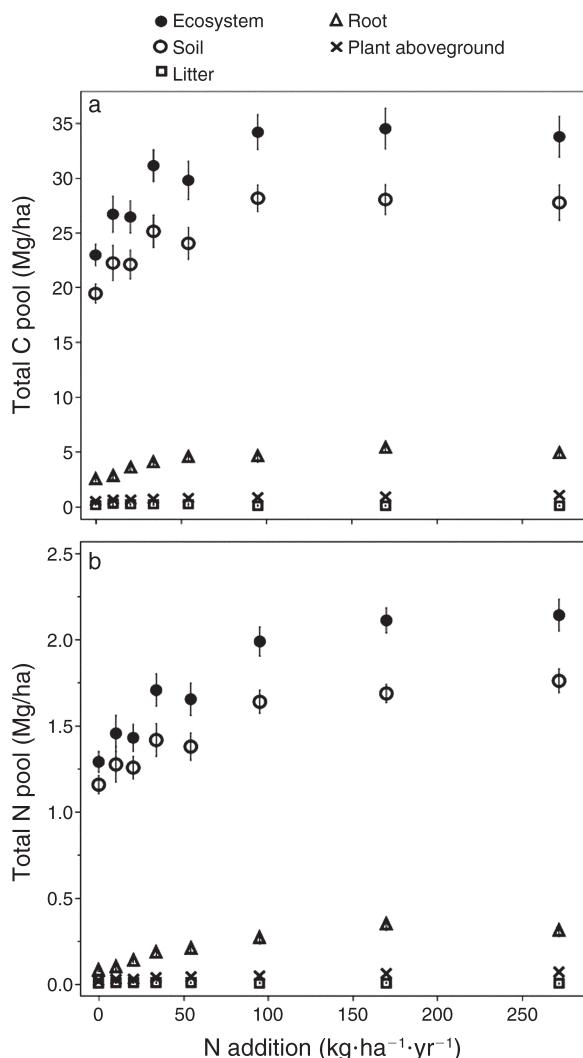
Fig. 1. Dependence of total ecosystem (a) C and (b) N pools on N addition rates. Total ecosystem C and N pools are the sum of four different pools: soil, roots, litter, and plant aboveground C and N pools all measured in 2009 (Appendix: Table A1). Error bars show ±SE and represent variation among plots receiving the same N addition treatment.

model assumptions whereas for all other analyses we used untransformed data. Based on previous studies (Tilman et al. 2006, Fornara and Tilman 2008) in similar grassland systems we performed multiple regressions where we included root mass, root C:N ratios, plant aboveground mass and plant aboveground C:N ratios as predictors and total ecosystem C and N pools, net changes in soil C and N sequestration as response variables. For each of the four-ecosystem pools (plant aboveground, litter, roots, and soil) we first calculated the difference in total C (and total N) between each fertilized plot and the average of the controls, and then divided this value by the cumulative amount of N added to that plot over 27 years. Thus we estimated (1) the units of C gained

in each ecosystem pool per units of N added (i.e., "C gain efficiency" expressed as: g C/g N added), and (2) the units of N retained in each ecosystem pool per units of N added (i.e., "N retention efficiency" expressed as: g N/g N added). Note that we explicitly refer to "N retention" and "C gain" because our grasslands did receive experimental N inputs but not C inputs. To determine the effects of fire and N additions on our ecosystem variables we used data for field B (54 plots) and performed multiple regression analyses where fire treatment was expressed as either present (1) or absent (0) from a plot. Data were analyzed using JMP version 9.0.0 (SAS Institute, Cary, North Carolina, USA).

RESULTS

N addition and C and N pools

We found that total ecosystem C and N pools have significantly increased across this 27-year period with increasing rates of N addition (Fig. 1a, b; Appendix: Table A1). Net changes in the C and N sequestration of soil mineral pools also increased significantly with increasing rates of N addition between 1982 and 2009 (Appendix: Fig. A1, Table A1). Greater C and N accrual occurred despite significantly lower C gain efficiency and N retention efficiency at higher N addition rates (Fig. 2a, b).

Root mass and net soil C sequestration

Net soil C sequestration was positively related to root mass ($P < 0.0001$, $F_{4, 106} = 19.9$; overall $F_{4, 106} = 19.1$, $R^2 = 0.42$; Fig. 3a) but negatively related to root C:N ratios ($P = 0.008$, $F_{4, 106} = 7.9$; Fig. 3b). We found that root mass still had significant positive effects (t ratio = 4.8, $P < 0.0001$) on net soil C sequestration in a type III multiple regression analysis, which also included N input rates as additional independent variable. Root mass also increased with N additions (Fig. 4a), whereas root C:N ratios (Fig. 4b) and root:shoot ratios (Fig. 4c) decreased at higher N addition rates.

N addition and plant community composition

N additions changed plant functional composition from native C_4 grass to introduced C_3 grass species (Fig. 4d, e), and also reduced plant diversity (Fig. 4f). The aboveground biomass of two C_3 grasses (*Agropyron repens*, *Poa pratensis*) accounted for 20% of total plant biomass at N deposition rates of 10 kg N·ha^{-1}·yr^{-1} and increased to 67% at rates of 54 kg N·ha^{-1}·yr^{-1}. Plant C:N ratios of these two species were 30.5 (*A. repens*) and 31.7 (*P. pratensis*) in control plots and decreased to 17.9 and 23.6 respectively in plots receiving 270 kg N·ha^{-1}·yr^{-1}. C_3 grass mass was positively related to root C pools (Appendix: Fig. A3a), and negatively related to root C:N ratios (Fig. A3b). Lower C:N root ratios of C_3 grasses may partly explain (together with direct soil N inputs) the higher soil N mineralization (Fig. A3c) associated with high C_3 grass biomass.
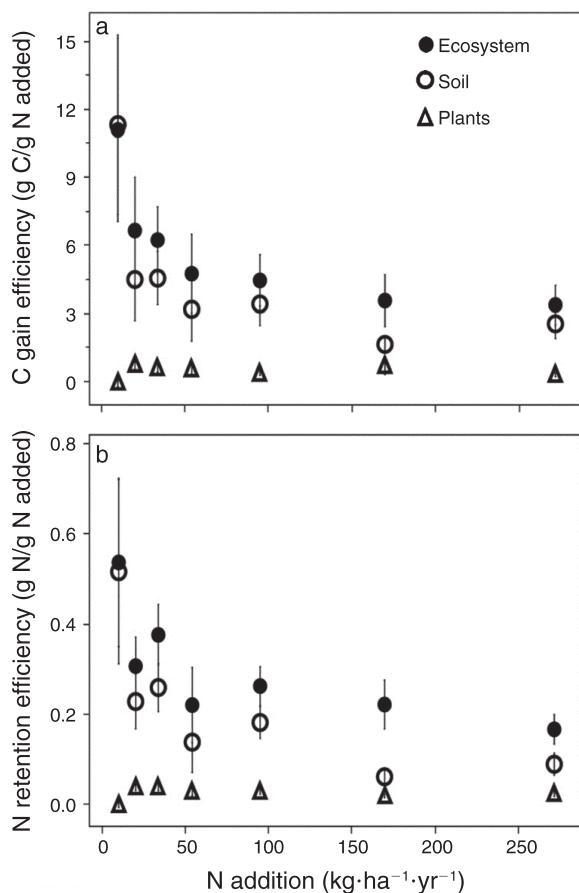
FIG. 2. The effect of different N addition rates on (a) C gain efficiency and (b) N retention efficiency of different ecosystem pools (i.e., whole ecosystem, soil, and plants). For simplicity, we averaged C and N values for roots, litter, and shoots. Error bars show ±SE and represent variation among plots receiving the same N addition treatment.

Frequent fires and C and N pools

Total soil C and N pools were not affected by frequent fire regimes in these grasslands. We found that 17 years of annual burning treatments (between 1992 and 2009) did not affect total ecosystem C pool (R^2 = 0.01, $F_{1,53}$ = 0.82, P = 0.36), total ecosystem N pool (R^2 = 0.001, $F_{1,53}$ = 0.08, P = 0.77; Fig. A4), total root mass (R^2 = 0.02, $F_{1,53}$ = 1.33, P = 0.25), net changes in soil C (R^2 = 0.03, $F_{1,53}$ = 1.9, P = 0.17) and in soil N sequestration (R^2 = 0.03, $F_{1,53}$ = 0.17, P = 0.67; Fig. A5).

DISCUSSION

Overall, our results show that at N addition rates of 10–20 kg N·ha^{-1}·yr^{-1} (i.e., common rates of N deposition across large regions worldwide; Bobbink et al. 2010), our prairie grasslands significantly increased C sequestration in mineral soils (>0.11 Mg C·ha^{-1}·yr^{-1} compared to unfertilized grasslands) and that the soil C accrual was even greater at higher N additions. This C increase occurred while N additions also reduced the soil

C gain efficiency (and soil N retention) of these grasslands, shifted species composition toward exotic C$_3$ grasses with low tissue C:N ratios, and decreased plant species diversity.

N-induced increases in soil C sequestration: potential mechanisms

We found that a potential mechanism responsible for soil C accrual in our grasslands was a N-induced increase in root mass. Roots are responsible for substantial C contributions to mineral soils (Trumbore and Gaudinski 2003), which mainly occur through root turnover and root decomposition processes but also through root exudations (Farrar et al. 2003). Previous studies show that total root mass was positively correlated with soil C sequestration (Fornara and Tilman 2008, Lu et al. 2011). Also a recent meta-analysis (Van Groenigen et al. 2006) shows that under elevated CO$_2$ levels root biomass significantly increased and contributed to greater soil C sequestration but only at N addition rates >30 kg N·ha^{-1}·yr^{-1}. This suggests
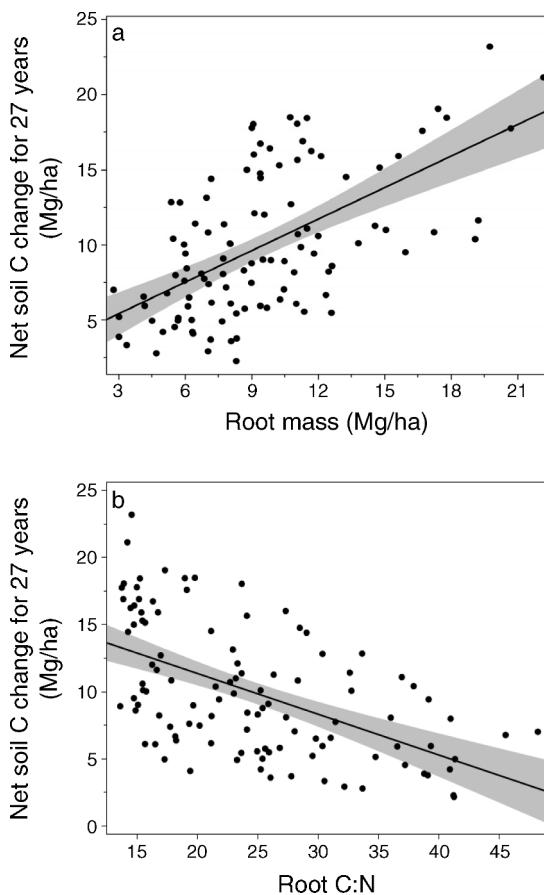

FIG. 3. Significant relationships between net soil C changes for 27 years (1982–2009) and (a) total root mass and (b) root C:N ratios, all measured in each of the experimental plots in 2009. Gray bands around regression lines represent 95% confidence intervals.
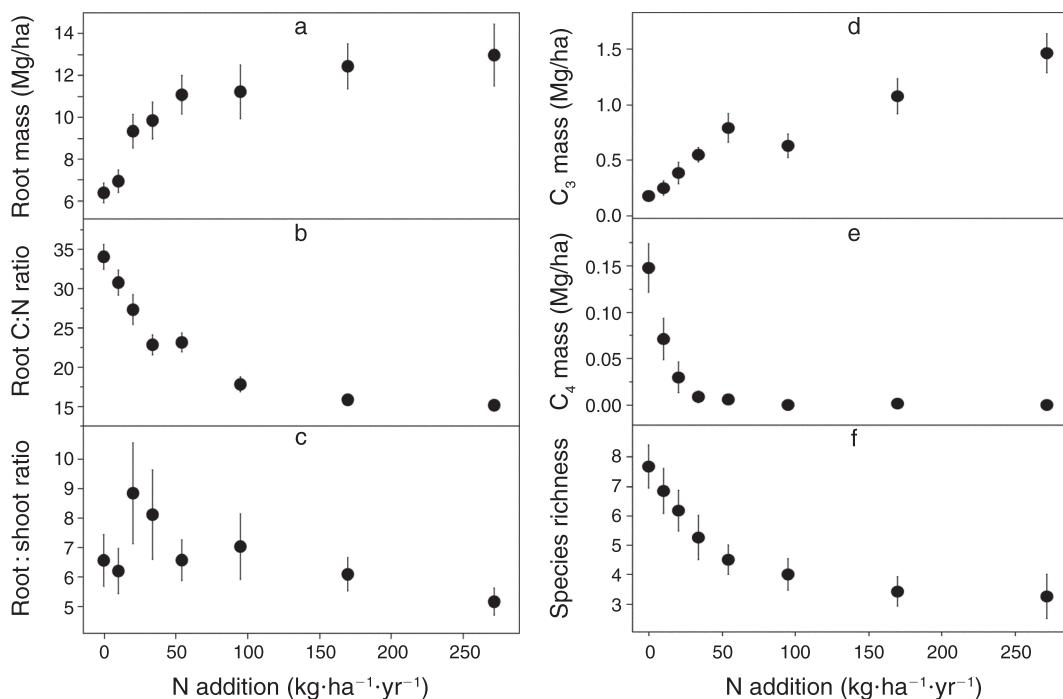
Reports

FIG. 4. Dependence of (a) root mass, (b) root C:N ratios, (c) root : shoot ratios, (d) C_3 grass aboveground biomass, (e) C_4 grass aboveground biomass, and (f) species richness on N addition rates. Error bars show ±SE and represent variation among plots receiving the same N addition treatment.

that N-induced increases in root mass may enhance soil C sequestration at increasing CO_2 levels, a response that would not be expected by progressive N limitation theory under low N availability (Luo et al. 2004).

In our grassland experiments it is not clear to what extent increases in root mass from exotic C_3 grasses were responsible for greater soil C sequestration. The findings of a recent experiment in semiarid grasslands (Macdougall and Wilson 2011) show a double increase of root production in communities invaded by the C_3 grass *Agropyron cristatus* but this was not clearly related to increases in soil C. By comparing the slopes of best-fit logarithm functions (see Appendix: Table A1) we found that the rate of increase in soil mineral C was actually faster than the rate of increase in root C. Thus it seems that other synergistic mechanisms may have contributed to the soil C accrual. For example, recent findings from the same grassland experiments at Cedar Creek (Ramirez et al. 2010, 2012) show predictable and consistent changes in the structure of bacterial communities across similar gradients of N fertilization. These changes are associated with increases in microbes that are less capable of decomposing recalcitrant soil C pools, thus leading to increases in C sequestration (Ramirez et al. 2012). Previous studies also suggest that N inputs can decrease microbial mineralization of soil organic pools, effectively slowing down decomposition (Janssens et al. 2010, Liu and Greaver 2010, Cusack et al. 2011).

Our data also show that high rates of N addition changed community composition from native C_4 grass-

lands to exotic C_3 grasslands, a functional shift that has possibly influenced root- and microbial-related processes. For example, greater root mass in N-enriched plots was associated with lower root C:N ratios (Fig. 4a, b). A higher decomposability of C_3 grass fine roots compared to C_4 grasses (Fornara et al. 2009) may have contributed to shifts in the soil microbial community toward faster-growing microbial communities, which may rely more on labile C sources (Ramirez et al. 2012).

N addition and soil C "saturation"

A critical question remains to what extent N-dependent soil C accrual may continue before other ecological mechanisms determine a soil C "saturation point." For example, C and N sequestration in our N-enriched soils may become limited by low soil C gains per unit of N added at higher N deposition rates. Thus we need to understand how net soil C gains vary at increasing N additions. Previous studies (Nadelhoffer et al. 1999, de Vries et al. 2006) suggest a "stoichiometric scaling" approach whereby net soil C gain is calculated on the basis of the soil N retention fraction multiplied by soil C:N ratio assuming that soil C:N ratios remain constant at increasing N additions rates. Soil C:N ratios, however, slightly decreased with N addition rates in our study (Appendix: Fig. A2). By comparing soil C:N ratios with soil C:N ratios "sequestered" (i.e., net soil C sequestration/net soil N sequestration for 27 years) we found that net soil C gain per unit of N added decreased significantly after 10 kg $N·ha^{-1}·yr^{-1}$, which suggests a

much reduced ability to store extra C per unit of N. It could also be that soil C sequestration is limited by high N losses, which could negatively affect soil organic formation. Fig. 2b suggests that soil N retention is >50% at N deposition rates of 10 kg N·ha^{-1}·yr^{-1} and drops to ~20% at deposition rates of 20 kg N·ha^{-1}·yr^{-1}.

N addition, soil C gains, and ecosystem services: a broader context

The unexpected soil C sequestration service provided by exotic C$_3$ grasslands is a novel finding of our study. These grasslands may store ~0.11 Mg C·ha^{-1}·yr^{-1} in mineral soils at N fertilization rates ranging between 10 and 20 kg N·ha^{-1}·yr^{-1}. These rates of soil C sequestration are similar or lower than those estimated in forest studies (e.g., 0.11–0.19 Mg C·ha^{-1}·yr^{-1}; see De Vries et al. 2006). However, at comparable rates of N addition, our grasslands gain much less C per unit of N input (i.e., 5 kg C/kg N) than do forests (i.e., >15 kg C/kg N; see Nadelhoffer et al. 1999, de Vries et al. 2006). A potential explanation for this could be the higher recalcitrance of forest litter (i.e., higher lignin content) and the negative effects by N addition on its decomposition (Janssens et al. 2010), thus leading to more soil C accumulation per unit of N added.

The presence of fire, which is a major "consumer" of litter in our grassland ecosystems, did not alter the positive effect of N addition on soil C sequestration. Because total root mass in these prairie communities was not significantly affected by the burning treatment ($F_{1,53} = 1.3$, $P = 0.25$), this may suggest that fire had not reduced plant C inputs belowground, suggesting that litter C inputs may have been small relative to C inputs from roots in these grassland ecosystems. It could also be possible that C stocks in mineral soils are resilient to fire disturbance.

Finally, in our study N additions simultaneously affected plant species diversity and soil C sequestration, which are both considered highly valuable from an "ecosystem service" perspective. We know from an experiment on similarly sandy soils in a nearby field at this research site that, without any added N, high diverse plant communities (i.e., 16 species mixtures) mainly because of greater root mass accumulation can store an additional ~0.28 Mg C·ha^{-1}·yr^{-1} relative to monocultures of these same species (Fornara and Tilman 2008). Our findings however, show that N addition despite decreasing plant diversity (Fig. 4f) also led to greater C sequestration in our grassland soils, suggesting that this ecosystem service can be delivered, albeit at a much lower rate, by low-diversity grassland systems. An important challenge remains to disentangle the underlying mechanisms responsible for soil C accrual and for the delivery of other ecosystem services in both (1) high-diversity grasslands and (2) the low-diversity nitrophilous grasslands being created by N deposition across different eco-regions worldwide.

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Supplemental Material

Appendix

Tables and graphs related to the analysis of potential N fertilization and fire effects on different environmental variables (*Ecological Archives* E093-194-A1).

Reports