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Reconciling multiple impacts of nitrogen enrichment on soil carbon: plant, microbial and geochemical controls

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Abstract

Impacts of reactive nitrogen (N) inputs on ecosystem carbon (C) dynamics are highly variable, and the underlying mechanisms remain unclear. Here, we proposed a new conceptual framework that integrates plant, microbial, and geochemical mechanisms to reconcile diverse and contrasting impacts of N on soil C. This framework was tested using long-term N enrichment and acid addition experiments in a Mongolian steppe grassland. Distinct mechanisms could explain effects of N on particulate and mineral-associated soil C pools, potentially explaining discrepancies among previous N addition studies. While plant production predominated particulate C changes, N-induced soil acidification strongly affected mineral-associated C through decreased microbial growth and pH-sensitive associations between iron and aluminum minerals and C. Our findings suggest that effects of N-induced acidification on microbial activity and geochemical properties should be included in Earth system models that predict ecosystem C budgets under future N deposition/input scenarios.

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Statement of authorship

SH and YB conceived the research. YB and DC designed and conducted the field experiment. CY and DC performed the lab analyses. CY, DC, SH, SJH and YB analyzed the data. CY, SJH, and SH wrote the manuscript with significant inputs from all other authors.

Data accessibility statement

We confirm that if our manuscript is accepted, the data supporting the results will be archived in an appropriate public repository.

Abstract

Impacts of reactive nitrogen (N) inputs on ecosystem carbon (C) dynamics are highly variable, and the underlying mechanisms remain unclear. Here, we proposed a new conceptual framework that integrates plant, microbial, and geochemical mechanisms to reconcile diverse and contrasting impacts of N on soil C. This framework was tested using long-term N enrichment and acid addition experiments in a Mongolian steppe grassland. Distinct mechanisms could explain effects of N on particulate and mineral-associated soil C pools, potentially explaining discrepancies among previous N addition studies. While plant production predominated particulate C changes, N-induced soil acidification strongly affected mineral-associated C through decreased microbial growth and pH-sensitive associations between iron and aluminum minerals and C. Our findings suggest that effects of N-induced acidification on microbial activity and geochemical properties should be included in Earth system models that predict ecosystem C budgets under future N deposition/input scenarios.

INTRODUCTION

Anthropogenic reactive nitrogen (N) inputs, largely caused by fossil-fuel combustion

and chemical fertilizer applications, have exceeded N inputs through all natural

processes over recent decades (Vitousek *et al.* 1997; Davidson 2009). Because of the

tight coupling between terrestrial carbon (C) and N cycles, the increased N in soil has

considerably altered terrestrial ecosystem processes such as plant productivity and

decomposition, that in turn affect ecosystem C dynamics (Reay *et al.* 2008; Liu &

Greaver 2010; Riggs & Hobbie 2016). Soils contain the largest near-surface reservoir

of organic C in terrestrial ecosystems (Jobbagy & Jackson 2000), and changes in soil

C in response to N enrichment may thus have substantial impacts on the net CO_2

exchange between the atmosphere and the biosphere.

The impact of N inputs on soil C storage has received considerable research

attention. In theory, N inputs can stimulate the production of plant biomass,

increasing C inputs to soil (Lebauer & Treseder 2008; Hyvönen *et al.* 2008; Pregitzer

et al. 2008; Fornara & Tilman 2012). However, many studies have shown that

increased net primary production does not always lead to net increases in C storage.

Neutral and negative effects of N inputs on soil C storage have also been reported

(Neff *et al.* 2002; Mack *et al.* 2004; Zeglin *et al.* 2007; Liu & Greaver 2010).

A key source of the inconsistent and conflicting results among studies may lie in

the differential responses of various soil C pools to N enrichments (Averill & Waring

2017). The total soil C stock can be represented by multiple operational pools with

different turnover times linked to physico-chemical protection mechanisms (Christensen 2001; Zimmermann *et al.* 2007). Researchers often separate total soil C into light and heavy fractions by density fractionation (von Lützow *et al.* 2007). Light fraction C consists of partially decomposed plant materials and by-products of decomposition (and potentially charred material), while heavy fraction C consists of mineral-sorbed C and a variable fraction of aggregate-occluded particulate C (Six *et al.* 2006; von Lützow *et al.* 2007). These density fractions represent mixtures of pools with differing turnover times (Torn *et al.* 2013; Hall *et al.* 2015), but nonetheless represent useful operational separations for understanding soil C cycling processes (Christensen 2001; Zimmermann *et al.* 2007).

Intriguingly, the effects of N addition on both light and heavy fraction C differ among studies. The impact of N addition on light fraction C has been reported to be both positive (Huang *et al.* 2011; Song *et al.* 2014) and negative (Neff *et al.* 2002; Cusack *et al.* 2011). Similarly, many field experiments have shown that N additions promoted soil C accumulation in mineral-associated fractions (Neff *et al.* 2002; Cusack *et al.* 2011; Huang *et al.* 2011), but contradicting results have also been reported (Bradford *et al.* 2008; Song *et al.* 2014; Riggs *et al.* 2015). Multiple microbial mechanisms have been proposed to explain these controversial results, such as changes in microbial extracellular enzyme activities, the decomposer community, or microbial carbon use efficiency under N additions (Fog 1988; Schimel & Bennett 2004; Waldrop *et al.* 2004; Riggs & Hobbie 2016), but the exact mechanisms remain

uncertain.

While N addition increases N availability, it can also cause soil acidification. This is due to increased nitrification and associated proton (H^+) production accompanied by leaching of base cations with nitrate (NO_3^-), as well as the biological uptake of ammonium (NH_4^+), which also releases H^+ (Fig. 1; Matson *et al.* 1999; Guo *et al.* 2010). At the global scale, soil pH decreases linearly with N addition (Tian & Niu 2015), consistent with an exponential increase in [H^+]. Soil acidification can strongly affect microbial metabolism. Addition of N tends to decrease microbial biomass (Liu & Greaver 2010; Treseder 2008) and a recent N addition experiment showed that soil acidification best explained observed decreases in microbial biomass and microbial respiration (Chen *et al.* 2016). Emerging evidence has also demonstrated that microbial residues derived from catabolism of light fraction C are important constituents contributing to mineral-associated C (Cotrufo *et al.* 2015; Liang *et al.* 2017). Thus, regardless of increased plant litter inputs, N-induced acidification may suppress microbial decomposition and hence decrease the pool of microbial biomass that serves as precursors for C inputs to heavy fractions (i.e., organo-mineral associations).

In addition, increased leaching of base cations (e.g., K^+, Mg^{2+} and Ca^{2+}) and greater solubility of iron (Fe) and aluminum (Al) phases that co-occur with acidification could also play a key role in altering the sorption and co-precipitation reactions that affect mineral-associated C. Some studies found that the increased

solubility of hydrolyzing cations (e.g., Fe^{3+} and Al^{3+}) at low pH could increase the

stabilization of C in organo-metal associations thought to protect C from microbial

decomposition (Gu *et al.* 1994; Mueller *et al.* 2012). Most organo-metal complexes

associate with the heavy, mineral-associated fraction (Kaiser & Guggenberger 2007).

However, losses of polyvalent cations—especially Ca^{2+}—can promote the release of

organic C adsorbed to mineral surfaces through polyvalent cation bridging (von

Lützow *et al.* 2006). Thus, the effects of N additions on soil C pools depend not only

on direct effects on plant productivity and microbial activity, but also on soil

geochemical properties mediated by N-induced soil acidification.

The interrelated responses of plants, microbes and minerals to N inputs likely

result in contrasting effects on different soil C pools. Although a model simulation

recently showed that microbial physiology, mineralogy, and acidity may co-affect the

impact of N inputs on soil C dynamics (Averill & Waring 2017), no experimental

studies to date have directly demonstrated these interactions in field. In this study, we

propose and test a conceptual framework linking multiple plant, microbial, and

geochemical mechanisms to explain the variable and potentially counteracting effects

of N addition on soil C pools (Fig. 1). First, N inputs directly increase N availability

for plants and soil microbes. In N-limited ecosystems, this increase in soil N

availability could promote plant growth and increase production of plant litter

(Lebauer & Treseder 2008). Although the increase in quantity of plant biomass could

increase light fraction C, this would depend on concomitant effects of N on litter

decomposition rates. Therefore, the change of soil labile C (largely particulate C) in response to N addition depends on both production of plant biomass and soil microbial activity. Second, N inputs can also result in soil acidification (Guo *et al.* 2010; Chen *et al.* 2016), thereby suppressing microbial decomposition, increasing the solubility of Fe and Al oxides, and enhancing leaching of base cations such as Ca^{2+}. Thus, acidification may disrupt C that is sorbed via Ca bridging, while simultaneously increasing pools of C co-precipitated with Fe and Al phases. We hypothesize that the relative strengths of these biotic and abiotic responses will determine the net effect of N addition on organo-mineral associations.

Taking advantage of a long-term N enrichment experiment initiated in 1999, we examined the effects of a gradient on N inputs on soil C pools in a Mongolian steppe grassland. This biome forms part of the Eurasian Steppe, extending over 8000 km from north-eastern China to Hungary (Bai *et al.* 2007). To assess impacts of N-mediated acidification, an acid addition experiment was conducted adjacent to the N enrichment experiment to generate comparable decreases in soil pH resulting from N inputs (Chen *et al.* 2016). This allows us to compare the effects of N-induced soil acidification and direct acid additions on soil C pools. Our objectives were to 1) investigate the effects of long-term reactive N inputs on various C pools with an emphasis on reconciling impacts of N on plant productivity, microbial decomposition, and C partitioning within and among density fractions, and 2) ascertain the role of N-induced acidification in regulating soil C dynamics.

MATERIAL AND METHODS

Study site

This study was conducted at the Inner Mongolia Grassland Ecosystem Research
Station of the Chinese Academy of Sciences, which is located in the Xilin River Basin
of Inner Mongolia, China (43°38' N, 116°42' N and 1250 m a.s.l.). The semi-arid
continental climate characterizes this steppe ecosystem. The mean annual temperature
is 0.3 °C, with mean monthly temperatures ranging from -21.6 °C in January to
19.0 °C in July. Mean annual precipitation is 346.1 mm, with about 80 % falling in
the growing season (June–August). The soil at this site is classified as dark chestnut
(Calcic Chernozem according to ISSS Working Group RB, 1998), with a loamy-sand
texture. The clay, silt and sand contents were 2.22 %, 17.60 % and 80.18 %,
respectively (Bai *et al.* 2010).

Field experiments

The N enrichment experiment was established in 1999. There were six levels of N
addition rate: 0, 1.75, 5.25, 10.50, 17.5, and 28.0 g N m^{-2} yr^{-1}. Fifty-four plots each
measuring 5 × 5 m were arranged in a randomized block design including nine
replicate blocks and separated by 1 m buffers. Reactive N was added as commercial
pelletized NH_4NO_3 fertilizer at the middle of the growing season (July 1 − 5). To
ensure that N was the primary limiting nutrient, each plot also received P (10 g P_2O_5
m^{-2} yr^{-1}), S (0.2 mg m^{-2} yr^{-1}) and trace elements (Zn: 190 μg m^{-2} yr^{-1}, Mn: 160 μg m^{-2}

yr^{-1}, B: 31 µg m^{-2} yr^{-1}). Because these soils were rich in K, Mg, Ca and Fe, these four

nutrients were not added in this experiment (Bai *et al.* 2010). We added P and other

micronutrients at a constant level across N addition treatments to ameliorate co-

limitation by these nutrients in our system. This should be noted when comparing our

results to other N addition experiments where limitation by multiple nutrients may

have occurred, thus influencing the plant and microbial responses to N addition.

To assess the potential direct effects of soil acidification on soil organo-mineral

associations, a complementary acid addition experiment was established near the N

enrichment experimental site in 2009 to generate comparable degrees of soil

acidification resulting from N inputs (Chen *et al.* 2016). There were seven levels of

acid addition: 0, 2.76, 5.52, 8.28, 11.04, 13.80 and 16.56 mol H$^+$ m^{-2} yr^{-1} (a detailed

description is provided in Materials and Methods S1).

Plant and soil sampling

In this study, we chose four treatments from the N enrichment experiment (0, 1.75,

10.50 and 28.0 g N m^{-2} yr^{-1}) and four treatments from the acid addition experiment (0,

2.76, 11.04 and 16.56 g mol H$^+$ m^{-2} yr^{-1}) for plant and soil sampling. In August 2014,

above-ground vegetation was collected in a 0.5 m × 0.5 m quadrat to determine

above-ground plant biomass in each plot of the N enrichment experiment and acid

addition experiment. After the above-ground biomass was sampled, three soil cores

(6.5 cm diameter and 0–20 cm depth) were collected in each plot to determine below-

ground plant biomass. Then four soil cores (2 cm diameter, 0–20 cm depth) were

randomly collected from each selected plot and combined to form one composite soil

sample. After roots and rocks were removed, the field moist soil was passed through a

2 mm mesh sieve and thoroughly mixed. The fresh soil was used for determination of

microbial parameters. Air-dried samples were used for determination of

physicochemical properties (Materials and Methods S1).

Determination of soil microbial biomass and microbial activity

Soil microbial biomass was estimated using the chloroform fumigation-extraction

method (Vance *et al.* 1987). Breifly, 12.5 g of fresh soil was fumigated with ethanol-

free chloroform for 48 h. Both fumigated and non-fumigated soils were extracted with

50 mL 0.5 M K_2SO_4 solution and the extracts were measured on the TOC analyzer to

determine organic C concentrations. The measured organic C was converted to

microbial biomass C using a conversion factor $k_{ec} = 0.33$ (Mo *et al.* 2008). Soil

microbial activity was measured by determining microbial respiration during an 11-

week incubation period. The respired CO_2 was determined by an incubation-alkaline

absorption method (Hu & vanBruggen 1997). The cumulative C lost by respiration

during incubation was calculated following the formula described by Liu *et al.* (2009).

Density fractionations and their C contents

The bulk soil was separated into two operationally defined soil fractions, a free light

fraction and a heavy fraction (> 1.8 g cm^{-3}), following a density fractionation

technique. Then, the separated soil fractions were dried in an oven at 80 °C and then

ground to a homogenized fine powder for organic C analysis using the CN analyzer

(Materials and Methods S1).

Determination of Ca-bound C, Fe-bound C, and Fe and Al phases

The heavy fraction soil samples were sequentially extracted with 0.5 M Na_2SO_4 and

dithionite-citrate-bicarbonate (DCB; soil:solution ratios of 1:50 for both extractions)

to separate organic C associated with Ca bridges and Fe mineral phases, respectively.

Because water soluble and weakly-exchangeable organic matter were removed from

soil colloids during the NaI density separation and subsequent rinses, we interpret C

released during Na_2SO_4 extraction as predominantly representing organic C associated

with Ca bridges (Xu & Yuan 1993). These solutions were measured on the TOC

analyzer. Following Na_2SO_4 extraction, the residual soil was extracted in DCB to

release Fe-associated organic C (Materials and Methods S1).

Determination of soil total crystalline and short-range-ordered minerals

Total mineral masses of short-range-ordered (SRO) and crystalline phases were

estimated by sequential chemical extractions of the heavy soil fractions (Chadwick *et*

al. 2003). The extraction procedures were as follows: (1) Organic matter was removed

with hydrogen peroxide and then the residual soil was rinsed and air dried. (2) The

residue was extracted using 0.275 M ammonium oxalate (pH 3.25) in the dark and

non-crystalline minerals were determined by recording weight loss after extraction.

(3) The residue from the ammonium oxalate dissolution was extracted by the DCB

method described above and weighed to determine crystalline mineral content. The

contents of SRO and crystalline minerals were expressed on a total soil basis.

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Statistical analyses

First, we used a linear mixed effects model to assess the impacts of N or acid additions on response variables, with N or acid treatments as fixed effects and blocks as a random effect. Then, one-way ANOVA with Duncan's multiple-range tests was performed across all response variables to compare the effects of N or acid additions. Second, we performed linear regression to analyze the responses of soil microbial biomass C, CO_2 efflux, total C in heavy fraction, Ca-bound C and Fe-bound C to soil pH. We also performed linear regression to analyze the responses of organic C in light fraction to plant biomass for N enrichment and acid addition experiment. In addition, ANCOVA was performed to estimate the slopes of these linear regressions and compare their differences between N enrichment experiment and acid addition experiment. All statistical analyses were performed using R version 3.3.3.

A structural equation modelling (SEM) approach was used to test a conceptual model for N enrichment impacts on soil particulate C and mineral-associated C (Fig. 1). We used principal component analysis to create a multivariate index for NH_4^+-N and NO_3^--N. The first principal component, which explained 91.0 % of the total variance, was used in the subsequent SEM analysis. The SEM analysis was performed with the *lavaan* package (Rosseel 2012) in R version 3.3.3 using the maximum likelihood estimation method. Several tests were used to assess model fit: the χ^2-test, comparative fit index (CFI), and root square mean error of approximation (RMSEM).

RESULTS

Soil abiotic properties, plant biomass and microbial biomass and respiration

Both N inputs and acid addition significantly decreased soil pH, with similar

decreases evident in both experiments ($P < 0.05$, Table S1). However, they had

contrasting effects on several C and N metrics. While N inputs increased soil NO_3^--N,

NH_4^+-N, total organic C, and total N, acid additions reduced all of these metrics ($P <$

0.05, Table S1). Neither N addition nor acid addition had significant impacts on

dissolved organic C (Table S1).

Addition of N significantly increased both above-ground and below-ground plant

biomass ($P < 0.05$, Fig. 2a, b), but significantly reduced microbial respiration and

microbial biomass C ($P < 0.05$, Fig. 3a, b). Acid additions had similar effects as N

additions on microbial respiration and microbial biomass C ($P < 0.05$, Fig. 3c, d), but

had no effects on plant biomass (Fig. 2c, d). Specifically, the cumulative microbial

respiration and microbial biomass C decreased with soil pH for both experiments, and

the slopes and intercepts of the linear regressions between pH and the two microbial

parameters did not significantly differ between the two experiments, except for the

intercepts between pH and microbial respiration ($P < 0.05$, Fig. 3e, f).

Soil organic C in different fractions

Addition of N significantly increased light fraction C ($P < 0.05$, Fig. 4a), while acid

addition significantly decreased light fraction C ($P < 0.05$, Fig. 4b). In the N

enrichment experiment, increases in above-ground and below-ground plant biomass

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explained 51 % and 39 % of the variation in light fraction C, respectively (Fig. 4c, d). In comparison, plant biomass had no significant effect on light fraction C in the acid addition experiment (Fig. 4c, d).

Both N inputs and acid addition significantly reduced the content of heavy fraction C and Na_2SO_4-extractable C (interpreted as Ca-bound C), but increased dithionite-extractable (interpreted as Fe-bound) C ($P < 0.05$, Fig. 5a–f). Linear regressions showed that decreased soil pH was significantly related to decreases in total heavy fraction C and the Ca-bound component of heavy fraction C, and increases in Fe-associated component of heavy fraction C, for both experiments (Fig. 5g–i). The slopes of the linear regressions between pH and these three response variables (heavy fraction C, Ca-bound C, and Fe-associated C) did not significantly differ between the N addition and acid addition experiments (Fig. 5g–i).

Geochemical characteristics

Neither N nor acid addition had significant impacts on DCB-extractable Fe and Al, SRO Fe and Al, total SRO minerals, and crystalline minerals (Table S1; Fig. S1). However, both N and acid additions significantly increased pyrophosphate-extractable Fe and Al concentrations ($P < 0.05$, Table S1), and decreased extractable Na^+, K^+, Mg^{2+}, Ca^{2+}, and Mn^{2+} concentrations ($P < 0.05$, Fig. S2), consistent with our conceptual framework (Fig. 1).

Controls on light and heavy fraction C in the N addition experiment

The optimal SEM model implied by our data supported most of the hypothesized

relationships among biogeochemical variables and soil C fractions (Fig. 6). Addition

of N directly induced changes in soil N availability and pH, explaining 93 % and

94 % of the total variance in these two variables, respectively (Fig. 6). Soil N

availability directly explained 73 % of the variance in above- and belowground

biomass, which in turn was associated with increased particulate organic C ($R^2 =$

49 %). However, the decreased soil pH resulting from N addition (as opposed to N

availability per se) was the optimal predictor for several response variables.

Decreased pH was associated with decreases in microbial biomass and Ca-associated

C, and increased Fe-associated C (R^2 values between 56 and 74 %). These findings

were in accordance with the results of the simple linear regressions described above

(Fig. 3f; Fig. 5h, i), and indicated that soil acidification induced by N addition directly

impacted microbial metabolism and mineral-associated C. In addition, particulate

organic C had a direct negative relationship with mineral-associated organic C, while

soil microbial biomass, and Fe- and Ca-associated C were not significantly related to

mineral-associated organic C.

DISCUSSION

Our study showed that although long-term N inputs significantly increased plant litter

production and bulk soil organic C, they significantly reduced heavy fraction C

content. Soil pH showed a strong, linear decrease with N addition, consistent with

global observations (Tian & Niu 2015). Consistent with our proposed conceptual

framework, our results suggested that the reduced heavy fraction C was largely

induced by soil acidification following N addition, which was confirmed by our

complementary acid addition experiment and linear regressions (Fig.5a, d, g). In

addition, our study also demonstrated that N-induced soil acidification led to

significant increased content of Fe-bound C and reduced content of Ca-bound C.

Together, these findings suggest that soil acidification may control impacts of N on

soil C dynamics via contrasting biological and geochemical mechanisms.

Nitrogen enrichment increased total soil C and light fraction C pools

Results from our study showed that long-term N additions enhanced total soil

organic C by increasing the C mass in the light fraction (Table S1). Enhancement of

soil organic C by N fertilization has been reported in many previous studies (e.g.

Fornara & Tilman 2012; Yue *et al.* 2016) and has been largely attributed to N

stimulation of plant growth and subsequent organic C inputs via aboveground

residues and roots (Lebauer & Treseder 2008; Liu & Greaver 2010). We also found

that N addition significantly increased light fraction C stocks (Fig. 4a), in contrast to

Neff *et al.* (2002) and Cusack *et al.* (2011) who found that N additions did not affect

light fraction C and reduced light fraction C, respectively. Previous studies proposed

that enhanced decomposition of light fraction C with added N resulted from

increasing labile C inputs to soils (i.e., the priming effect), alleviating N limitation of

microbes, and/or facilitating the production of extracellular enzymes (Schimel &

Bennett 2004; Cusack *et al.* 2011; Riggs *et al.* 2015). In our study, N enrichment

increased both above- and below-ground biomass, consistent with the findings of a

meta-analysis (Xia & Wan 2008). Our statistical models (Fig. 6) implied that the

increase in light fraction C with N addition was largely due to increased plant biomass

inputs, as evidenced by a strong positive relationship between above- and below-

ground biomass and light fraction C (Fig. 4c, d). In the acid addition experiment, we

found a small but significant decrease in light fraction C with increasing acidity even

though plant biomass was unaffected in 2014. This may have resulted from a slight

suppression of plant productivity under acid addition that occurred earlier in the

experiment (Chen *et al.* 2013).

Nitrogen enrichment decreased the heavy fraction C pool

Contrary to increases in heavy fraction C observed in some other N addition studies

(Neff *et al.* 2002; Huang *et al.* 2011; Cusack *et al.* 2011), we found that N additions

significantly reduced heavy fraction C (Fig. 5a). The net amount of heavy fraction C

depends on the balance between newly transformed C from faster-cycling pools and

decomposition losses from the existing heavy fraction C. The majority of heavy

fraction C in temperate ecosystems often cycles over decadal timescales (Baisden *et*

al. 2002; Koarashi *et al.* 2012), consistent with our observations of significantly

altered heavy fraction C stocks following 15 years of N addition. Since N additions

had consistently increased plant production and litter inputs over time in our study site

(Bai *et al.* 2010; Chen *et al.* 2015; Chen *et al.* 2016), the decreased heavy fraction C

was likely due to reduced organic C inputs to the decadal-cycling heavy fraction pool

and/or increased decomposition losses of the existing heavy fraction C. Therefore, we propose that at least two mechanisms contributed to this decrease, consistent with our statistical models and comparisons between findings from the N addition and acid addition experiments.

First, decreases in microbial biomass were statistically associated with strong decreases in heavy fraction C (Fig. S3), consistent with the importance of microbial detritus as a key input to mineral-associated C pools (Cotrufo *et al.* 2013, Lehmann & Kleber 2015). It has long been noted that N can alter microbial enzyme production, potentially contributing to suppression of lignin-degrading enzymes while potentially enhancing carbohydrate catabolism (Fog 1988). We found that suppression of microbial biomass and respiration under N addition were strongly linked to decreased pH, as confirmed by the similar slopes of the linear regressions between pH and microbial respiration and biomass for the N enrichment and acid addition experiments (Fig. 3e, f). Addition of N had an additional suppresive effect on microbial respiration indicated by differing regression intercepts between the N and acid addition experiments (Fig. 3e), but intercepts for microbial biomass were similar (Fig. 3f). These findings strongly suggested that acidification, not N addition per se, drove the decrease in microbial biomass with pH. Furthermore, the SEM analyses were consistent with direct effects of acidification following N inputs, rather than the increased soil N availability itself, in suppressing microbial biomass (Fig. 6).

Microbes can further influence mineral-associated C fluxes by affecting soil

structure. Fungal hyphae and plant roots often promote aggregate formation, which

can further protect mineral-associated C from microbial attack (Wilson *et al.* 2009).

At our experimental site, recent studies observed that long-term N additions

significantly reduced mycorrhizal and total fungal biomass (Chen *et al.* 2016; Wei *et

al.* 2013). Together, these results indicate that N-induced reduction in microbial

biomass and activities, likely mediated by acidity, could reduce both C inputs to the

heavy fraction and their physical protection via aggregation.

The second likely mechanism contributing to decreased mineral-associated C was

the replacement of Ca^{2+} by H^+ and the subsequent leaching of Ca^{2+} along with nitrate,

and release of C stabilized via cation bridging. To the best of our knowledge, this

mechanism has not previously been addressed in the context of N impacts on

ecosystem C dynamics, but is likely to be important in many soils with circumneutral

pH where significant C is stabilized via Ca bridging (Oades 1988). The vast majority

of studies have so far attempted to explain the impact of N additions on soil C

dynamics by focusing on litter inputs and microbial decomposition (Liu & Greaver

2010; Janssens *et al.* 2010; Lu *et al.* 2011) rather than geochemical mechanisms of C

stabilization. For example, Cusack *et al.* (2011) attributed increased heavy-fraction C

in response to N addition to decreases in microbial oxidative enzymes. However,

long-term N additions can induce a series of changes in soil physical and chemical

properties, particularly soil acidity (Guo *et al.* 2010), which may directly affect

mineral-organic C interactions. Emerging evidence has recently shown that protection

of organic C from microbial attack, rather than intrinsic organic matter recalcitrance,

plays a dominant role in maintaining soil organic C stability (Schmidt *et al.* 2011;

Lehmann & Kleber 2015). Organo-mineral complexes can act as nuclei to form

microaggregates that are key to organic C protection (Vogel *et al.* 2014; Yu *et al.*

2017). Soil acidification can affect the speciation and solution activities of soil

minerals and cations that interact with organic carbon (Gu *et al.* 1994; Scheel *et al.*

2007; Yu *et al.* 2017). The soil at our study site is alkaline with sandy texture and low

buffering capacity (Bai *et al.* 2010). Interaction with clay minerals by polyvalent

cation bridges, especially Ca^{2+} and Mg^{2+} ions, is an important stabilization mechanism

for organic C across a wide spectrum of neutral and alkaline soils (Oades 1988; von

Lützow *et al.* 2006; Setia *et al.* 2014). Our study showed that both N and acid

additions led to polyvalent cation depletion and reduced Ca-bound C stocks (Fig. 5c,

f; Fig. S2; Fig. 6). Losses of these cations are often accompanied with soil

acidification. However, the solubility of most Fe mineral phases increases as pH

decreases. The observation of increasing Fe-bound C along with decreasing pH

indicated that acidification shifted organic-mineral associations from Ca bridging to

Fe-bound C (Fig. 5b, c).

   The soils in our study system have a sandy texture with comparatively low reactive

Fe and Al content (Table S1) relative to weathered soils with more abundant silt and

clay-sized fractions, but this should not affect the direction of the underlying

responses of Ca- and Mg-bound, or Fe and Al-associated C pools to N-induced

acidification. In fact, smaller total concentrations of metal-associated C phases may have enabled us to detect treatment effects on these pools over decadal timescales. We expect pH-sensitive organomineral interactions to be quantitatively even more important to total soil C in soils with greater reactive metal stocks. The tradeoff between increased Fe-bound C versus the losses in Ca-bound C and other C-mineral complexes resulting from soil acidification is likely to be broadly applicable among soils, but the absolute magnitudes will depend on the initial pH value, pH buffering capacity, and textural and elemental composition of a given soil.

For example, in acidic soils where Ca-bound C is unimportant, N-induced acidification could potentially increase total mineral-associated C stocks as a consequence of increased Fe solubility and the formation of Fe-C co-precipitates. This presents a plausible hypothesis for interpreting increases in mineral-associated C stocks following N addition to acidic, Fe-rich soils with clay texture (Cusack *et al.* 2011). Taken together, our results suggest that the loss of an organic C fraction chemically bound with polyvalent cation bridges (such as Ca-organic C associations) caused by N-induced acidification, along with reduced microbial C inputs, may function as major mechanisms by which N inputs reduce heavy fraction C in neutral and alkaline soils.

Implications for grassland ecosystem C sequestration

Understanding the mechanisms controlling soil organic C storage in response to N deposition is important for accurately predicting ecosystem C sequestration and/or

potential C losses accompanying increased anthropogenic N inputs. Results from our study showed that although reactive N inputs significantly increased bulk soil organic C and light fraction C content, they significantly reduced the stock of mineral-associated C. We suggest that this resulted from two pathways. First, soil acidification resulting from reactive N inputs may significantly reduce the heavy fraction C formation via suppressing microbial degradation of plant litter, growth of microbial biomass, and thus the production of likely precursors of mineral-associated organic matter. Second, N-induced acidification can enhance losses of the existing heavy fraction C by disrupting mineral-C complexes, particularly those involving Ca^{2+}, a factor which was only partially counteracted by increased Fe-associated C formed under acidic conditions. Together, our results indicate that over the long-term, N-induced acidification may constrain C sequestration in ecosystems where Ca- or Mg-bound C dominates the mineral-bound C. These findings suggest that the impacts of N-induced soil acidification on microbial activity and geochemical properties linked to C stabilization should be included as important factors in Earth system models that predict ecosystem C budgets and ecosystem properties under future N deposition/input scenarios.

REFERENCES

Averill, C. & Waring, B. (2017). Nitrogen limitation of decomposition and decay:

How can it occur? *Glob. Chang. Biol.*, 1−11. doi: 10.1111/gcb.13980.

Bai, Y., Wu, J., Clark, C.M., Naeem, S., Pan, Q., Huang, J. *et al.* (2010). Tradeoffs and

thresholds in the effects of nitrogen addition on biodiversity and ecosystem

functioning: evidence from inner Mongolia Grasslands. *Glob. Chang. Biol.*, 16,

358−372.

Bai, Y., Wu, J., Pan, Q., Huang, J., Wang, Q., Li, F. *et al.* (2007). Positive linear

relationship between productivity and diversity: evidence from the Eurasian

Steppe. *J. Appl. Ecol.*, 44, 1023−1034.

Baisden, W.T., Amundson, R., Cook, A.C. & Brenner, D.L. (2002). Turnover and

storage of C and N in five density fractions from California annual grassland

surface soils. *Global Biogeochem. Cy.*, 16, 1117.

Bradford, M.A., Fierer, N., Jackson, R.B., Maddox, T.R. & Reynolds, J.F. (2008).

Nonlinear root-derived carbon sequestration across a gradient of nitrogen and phosphorous deposition in experimental mesocosms. *Glob*. *Chang*. *Biol*., 14, 1113−1124.

Chadwick, O.A., Gavenda, R.T., Kelly, E.F., Ziegler, K., Olson, C.G., Elliott, W.C. *et al*. (2003). The impact of climate on the biogeochemical functioning of volcanic soils. *Chem*. *Geol*., 202, 195−223.

Chen, D., Lan, Z., Bai, X., Grace, J.B. & Bai, Y. (2013). Evidence that acidification-induced declines in plant diversity and productivity are mediated by changes in below-ground communities and soil properties in a semi-arid steppe. *J*. *Ecol*., 101, 1322−1334.

Chen, D., Lan, Z., Hu, S. & Bai, Y. (2015). Effects of nitrogen enrichment on belowground communities in grassland: Relative role of soil nitrogen availability vs. soil acidification. *Soil Biol*. *Biochem*., 89, 99−108.

Chen, D., Li, J., Lan, Z., Hu, S. & Bai, Y. (2016). Soil acidification exerts a greater control on soil respiration than soil nitrogen availability in grasslands subjected to long-term nitrogen enrichment. *Funct*. *Ecol*., 30, 658−669.

Christensen, B.T. (2001). Physical fractionation of soil and structural and functional complexity in organic matter turnover. *Eur*. *J*. *Soil Sci*., 52, 345−353.

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H. *et al*. (2015). Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nat*. *Geosci*., 8, 776−779.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K. & Paul, E. (2013). The

Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant

litter decomposition with soil organic matter stabilization: do labile plant inputs

form stable soil organic matter? *Glob. Chang. Biol.*, 19, 988−995.

Cusack, D.F., Silver, W.L., Torn, M.S. & McDowell, W.H. (2011). Effects of nitrogen

additions on above-and belowground carbon dynamics in two tropical forests.

Biogeochemistry, 104, 203−225.

Davidson, E.A. (2009). The contribution of manure and fertilizer nitrogen to

atmospheric nitrous oxide since 1860. *Nat. Geosci.*, 2, 659−662.

Fog, K. (1988). The effect of added nitrogen on the rate of decomposition of organic

matter. *Biol. Rev.*, 63, 433−462.

Fornara, D.A. & Tilman, D. (2012). Soil carbon sequestration in prairie grasslands

increased by chronic nitrogen addition. *Ecology*, 93, 2030−2036.

Gu, B., Schmitt, J., Chen, Z., Liang, L. & Mccarthy, J.F. (1994). Adsorption and

desorption of natural organic matter on iron oxide: mechanisms and models.

Environ. Sci. Technol., 28, 38−46.

Guo, J.H., Liu, X.J., Zhang, Y., Shen, J.L., Han, W.X., Zhang, W.F. *et al.* (2010).

Significant acidification in major Chinese croplands. *Science*, 327, 1008−1010.

Hall, S.J., McNicol, G., Nataken, T. & Silver, W.L. (2015). Large fluxes and rapid

turnover of mineral-associated carbon across topographic gradients in a humid

tropical forest: insights from paired ^{14}C analysis. *Biogeosciences*, 12,

2471−2487.

Hu, S. & vanBruggen, A.H.C. (1997). Microbial dynamics associated with
multiphasic decomposition of ^{14}C-labeled cellulose in soil. *Microb*. *Ecol*., 33,
134−143.

Huang, Z.Q., Clinton, P.W., Baisden, W.T. & Davis, M.R. (2011). Long-term nitrogen
additions increased surface soil carbon concentration in a forest plantation
despite elevated decomposition. *Soil Biol*. *Biochem*., 43, 302−307.

Hyvönen, R., Persson, T., Andersson, S., Olsson, B., Ågren, G.I. & Linder, S. (2008).
Impact of long-term nitrogen addition on carbon stocks in trees and soils in
northern Europe. *Biogeochemistry*, 89, 121−137.

Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans,
R. *et al*. (2010). Reduction of forest soil respiration in response to nitrogen
deposition. *Nat*. *Geosci*., 3, 315−322.

Jobbagy, E.G. & Jackson, R.B. (2000). The vertical distribution of soil organic carbon
and its relation to climate and vegetation. *Ecol*. *Appl*., 10, 423−436.

Kaiser, K. & Guggenberger, G. (2007). Distribution of hydrous aluminium and iron
over density fractions depends on organic matter load and ultrasonic dispersion.
Geoderma, 140, 140−146.

Koarashi, J., Hockaday, W.C., Masiello, C.A. & Trumbore, S.E. (2012). Dynamics of
decadally cycling carbon in subsurface soils. *J*. *Geophys*. *Res*., 117, G03033.

Lebauer, D.S. & Treseder, K.K. (2008). Nitrogen limitation of net primary

productivity in terrestrial ecosystems is globally distributed. *Ecology*, 89, 371−379.

Lehmann, J. & Kleber, M. (2015), The contentious nature of soil organic matter. *Nature*, 528, 60−68.

Liang, C., Schimel, J.P. & Jastrow, J.D. (2017). The importance of anabolism in microbial control over soil carbon storage. *Nat*. *Microbiol*., 2, 17105.

Liu, L.L. & Greaver, T.L. (2010). A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecol*. *Lett*., 13, 819−828.

Liu, L.L., King, J.S., Booker, F.L., Giardina, C.P., Allen, H.L. & Hu, S.J. (2009). Enhanced litter input rather than changes in litter chemistry drive soil carbon and nitrogen cycles under elevated CO_2: a microcosm study. *Glob*. *Chang*. *Biol*., 15, 441−453.

Lu, M., Zhou, X.H., Luo, Y.Q., Yang, Y.H., Fang, C.M., Chen, J.K. *et al*. (2011). Minor stimulation of soil carbon storage by nitrogen addition: A meta-analysis. *Agr*. *Ecosyst*. *Environ*., 140, 234−244.

Mack, M.C., Schuur, E.A.G., Bret-Harte, M.S., Shaver, G.R. & Chapin, F.S. (2004). Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature*, 431, 440−443.

Matson, P.A., Mcdowell, W.H., Townsend, A.R. & Vitousek, P.M. (1999). The globalization of N deposition: ecosystem consequences in tropical environments. *Biogeochemistry*, 46, 67−83.

Mo, J., Zhang, W., Zhu, W., Gundersen, P., Fang, Y., Li, D. *et al*. (2008). Nitrogen

 addition reduces soil respiration in a mature tropical forest in southern China.

 Glob. Chang. Biol., 14, 403−412.

Mueller, K.E., Eissenstat, D.M., Hobbie, S.E., Oleksyn, J., Jagodzinski, A.M., Reich,

 P.B. *et al*. (2012). Tree species effects on coupled cycles of carbon, nitrogen, and

 acidity in mineral soils at a common garden experiment. *Biogeochemistry*, 111,

 601−614.

Neff, J.C., Townsend, A.R., Gleixner, G., Lehman, S.J., Turnbull, J. & Bowman, W.D.

 (2002). Variable effects of nitrogen additions on the stability and turnover of soil

 carbon. *Nature*, 419, 915−917.

Oades, J.M. (1988). The retention of organic matter in soils. *Biogeochemistry*, 5,

 35−70.

Pregitzer, K.S., Burton, A.J., Zak, D.R. & Talhelm, A.F. (2008). Simulated chronic

 nitrogen deposition increases carbon storage in Northern Temperate forests.

 Glob. Chang. Biol., 14, 142−153.

Reay, D.S., Dentener, F., Smith, P., Grace, J. & Feely, R.A. (2008). Global nitrogen

 deposition and carbon sinks. *Nat. Geosci.*, 1, 430−437.

Riggs, C.E. & Hobbie, S.E. (2016). Mechanisms driving the soil organic matter

 decomposition response to nitrogen enrichment in grassland soils. *Soil Biol.

 Biochem.*, 99, 54−65.

Riggs, C.E., Hobbie, S.E., Bach, E.M., Hofmockel, K.S. & Kazanski, C.E. (2015).

Nitrogen addition changes grassland soil organic matter decomposition. *Biogeochemistry*, 125, 203−219.

Rosseel, Y. (2012). lavaan: an R package for structural equation modeling. *J. Stat. Softw.*, 48, 1−36.

Scheel, T., Dörfler, C. & Kalbitz, K. (2007). Precipitation of dissolved organic matter by aluminum stabilizes carbon in acidic forest soils. *Soil Sci. Soc. Am. J.*, 71, 64−74.

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

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A. *et al.* (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, 478, 49−56.

Setia, R., Rengasamy, P. & Marschner, P. (2014). Effect of mono-and divalent cations on sorption of water-extractable organic carbon and microbial activity. *Biol. Fert. Soils*, 50, 727−734.

Six, J., Frey, S.D., Thiet, R.K. & Batten, K.M. (2006). Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Sci. Soc. Am. J.*, 70, 555−569.

Song, B., Niu, S., Li, L., Zhang, L. & Yu, G. (2014). Soil carbon fractions in grasslands respond differently to various levels of nitrogen enrichments. *Plant soil*, 384, 401−412.

Tian, D. & Niu, S. (2015). A global analysis of soil acidification caused by nitrogen addition. *Environ. Res. Lett.*, 10, 024019.

Torn, M.S., Kleber, M., Zavaleta, E.S., Zhu, B., Field, C.B. & Trumbore, S.E. (2013). A dual isotope approach to isolate carbon pools of different turnover times. *Biogeosciences*, 10, 8067−8081.

Treseder, K.K. (2008). Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecol. Lett.*, 11, 1111−1120.

Vance, E.D., Brookes, P.C. & Jenkinson, D.S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.*, 19, 703−707.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W. *et al*. (1997). Human alteration of the global nitrogen cycle: Sources and consequences. *Ecol. Appl.*, 7, 737−750.

Vogel, C., Mueller, C.W., Höschen, C., Buegger, F., Heister, K., Schulz, S. *et al*. (2014). Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. *Nat. Commun.*, 5, 2947.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E. *et al*. (2007). SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. *Soil Biol. Biochem.*, 39, 2183−2207.

von Lützow, M., Kogel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B. *et al*. (2006). Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions-a review. *Eur. J.*

Soil Sci., 57, 426−445.

Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., Gallo, M. & Lauber, C. (2004). Nitrogen

deposition modifies soil carbon storage through changes in microbial enzymatic

activity. *Ecol. Appl.*, 14, 1172−1177.

Wei, C., Yu, Q., Bai, E., Lü, X., Li, Q., Xia, J. *et al*. (2013). Nitrogen deposition

weakens plant-microbe interactions in grassland ecosystems. *Glob. Chang. Biol.*,

19, 3688−3697.

Wilson, G.W., Rice, C.W., Rillig, M.C., Springer, A. & Hartnett, D.C. (2009). Soil

aggregation and carbon sequestration are tightly correlated with the abundance of

arbuscular mycorrhizal fungi: results from long-term field experiments. *Ecol.*

Lett., 12, 452−461.

Xia, J. & Wan, S. (2008). Global response patterns of terrestrial plant species to

nitrogen addition. *New Phytol.*, 179, 428−439.

Xu, J. & Yuan, K. (1993). Dissolution and fractionation of calcium-bound and iron-

and aluminum-bound humus in soils. *Pedosphere*, 3, 75−80.

Yu, G., Xiao, J., Hu, S., Polizzotto, M.L., Zhao, F., Mcgrath, S.P. *et al*. (2017). Mineral

availability as a key regulator of soil carbon storage. *Environ. Sci. Technol.*, 51,

4960−4969.

Yue, K., Peng, Y., Peng, C., Yang, W., Peng, X. & Wu, F. (2016). Stimulation of

terrestrial ecosystem carbon storage by nitrogen addition: a meta-analysis. *Sci.*

Rep., 6, 19895.

Zeglin, L.H., Stursova, M., Sinsabaugh, R.L. & Collins, S.L. (2007). Microbial

   responses to nitrogen addition in three contrasting grassland ecosystems.

   Oecologia, 154, 349−359.

Zimmermann, M., Leifeld, J., Schmidt, M.W.I., Smith, P. & Fuhrer, J. (2007). Measured

   soil organic matter fractions can be related to pools in the RothC model. *Eur. J.*

   Soil Sci., 58, 658−667.

Figure Captions:

Figure 1 Proposed conceptual diagram of contrasting effects of N addition on soil

particulate organic C and organo-mineral associations. Black arrows refer to positive

effects, red arrows refer to negative effects, and dashed arrows refer to uncertain

effects. N addition increases soil N availability and then affects plant growth and soil

heterotrophic microbial biomass and decomposition activity. The changes in

particulate organic C depend on both production of plant biomass and microbial

decomposition. N addition can also cause soil acidification due to increased

nitrification and the subsequent leaching of base cations with nitrate (NO_3^-), as well as

due to biological uptake of ammonium (NH_4^+), which releases a proton (H^+). The

leaching of base cations can promote the release of organic C that adsorbs to mineral

surface through polyvalent cation bridging. Nitrogen-induced soil acidification can

suppress microbial decomposition and increase solubility of Fe and Al, that in turn

limit the transformation of plant litter into organo-mineral associations, and enhance

Fe/Al-associated C. Thus, C content in organo-mineral associations depends on the

combined effects of these biotic and abiotic processes.

Figure 2 Responses of above-ground and below-ground biomass to N enrichment (a, b) and acid addition (c, d).

Figure 3 Responses of cumulative CO_2 production and microbial biomass C to N enrichment (a, b), acid addition (c, d), and the relationship between these microbial parameters and soil pH for the two experiments (e, f). The slopes and intercepts of the linear regressions between pH and microbial respiration: $slope_N = 0.39 \pm 0.06$; $intercept_N = -0.96 \pm 0.35$; $slope_{acid} = 0.26 \pm 0.08$; $intercept_{acid} = 0.34 \pm 0.49$. The slopes and intercepts of the linear regressions between pH and microbial biomass C: $slope_N = 211.13 \pm 36.85$; $intercept_N = -929.52 \pm 225.57$; $slope_{acid} = 188.38 \pm 25.63$; $intercept_{acid} = -794.01 \pm 154.82$.

Figure 4 Responses of light fraction C to N enrichment (a), acid addition (b), and relationships between light fraction C and plant biomass for the two experiments (c, d).

Figure 5 Responses of heavy fraction C, Fe-bound C, and Ca-bound C to N enrichment (a–c) and acid addition (d–f), and relationships between the different C fractions and pH for the two experiments (g–i). The slopes of the linear regressions between between pH and heavy fraction C: $slope_N = 0.97 \pm 0.36$; $slope_{acid} = 0.73 \pm 0.25$. The slopes of the linear regressions between pH and Fe-bound C: $slope_N = -0.19 \pm 0.04$; $slope_{acid} = -0.16 \pm 0.05$. The slopes of the linear regressions between pH and Ca-bound C: $slope_N = 0.27 \pm 0.06$; $slope_{acid} = 0.23 \pm 0.04$.

Figure 6 Structural equation model (SEM) analysis of the effects of N inputs on soil particulate organic C (POC) and mineral-associated organic C (MAOC) via pathways of soil N availability and soil acidification. Results of the optimal model fitting: Chi-square (χ^2) = 30.637, P = 0.104, degree of freedom (df) = 22, comparative fit index (CFI) = 0.952, root square mean error of approximation (RMSEA) = 0.157. Numbers at arrows are standardized path coefficients. Arrow thickness represents the strength of the relationships. Black arrows indicate significant positive relationships and red arrows indicate significant negative relationships ($P < 0.05$). Additionally, R^2 values associated with response variables indicate the proportion of variation explained by relationships with other variables. MBC, microbial biomass carbon; Fe-C, iron bound carbon; Ca-C, calcium bound carbon. In this model, POC and MAOC indicate light fraction C and heavy fraction C, respectively.
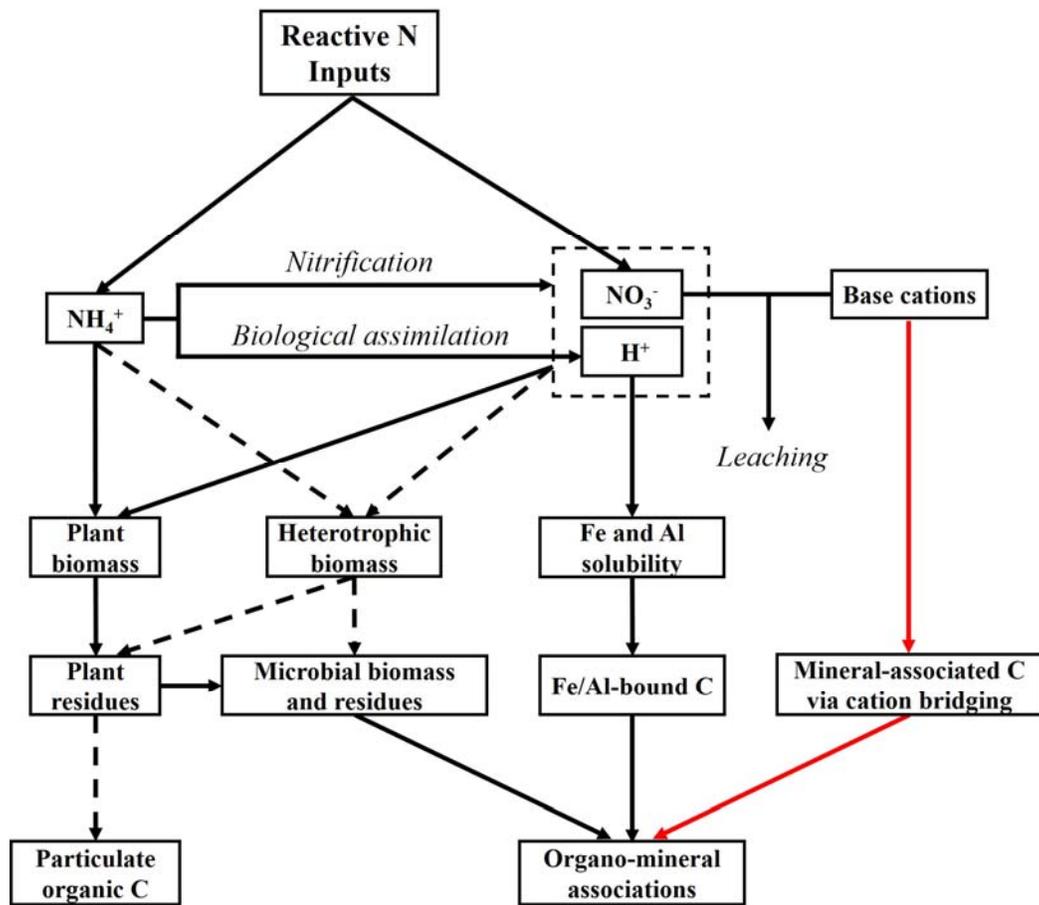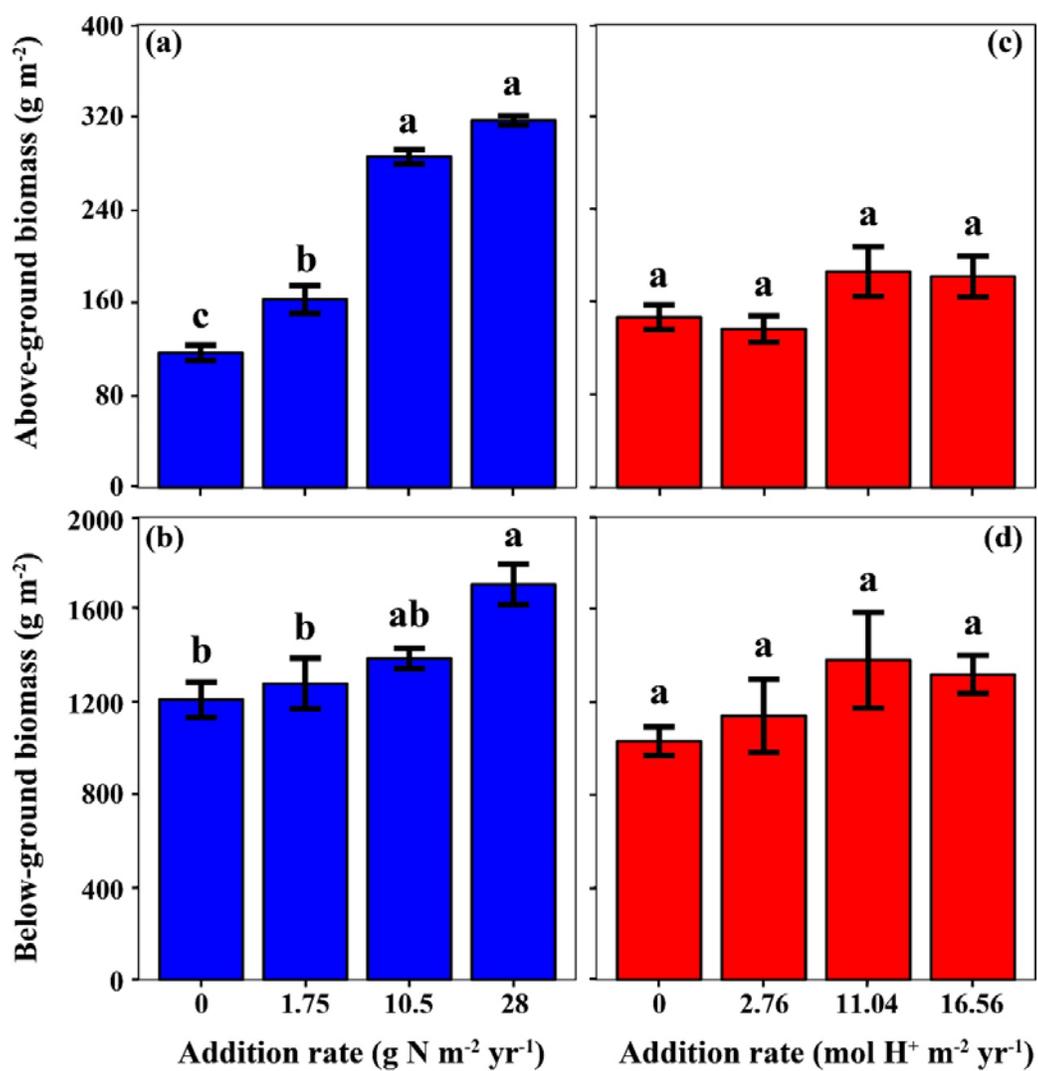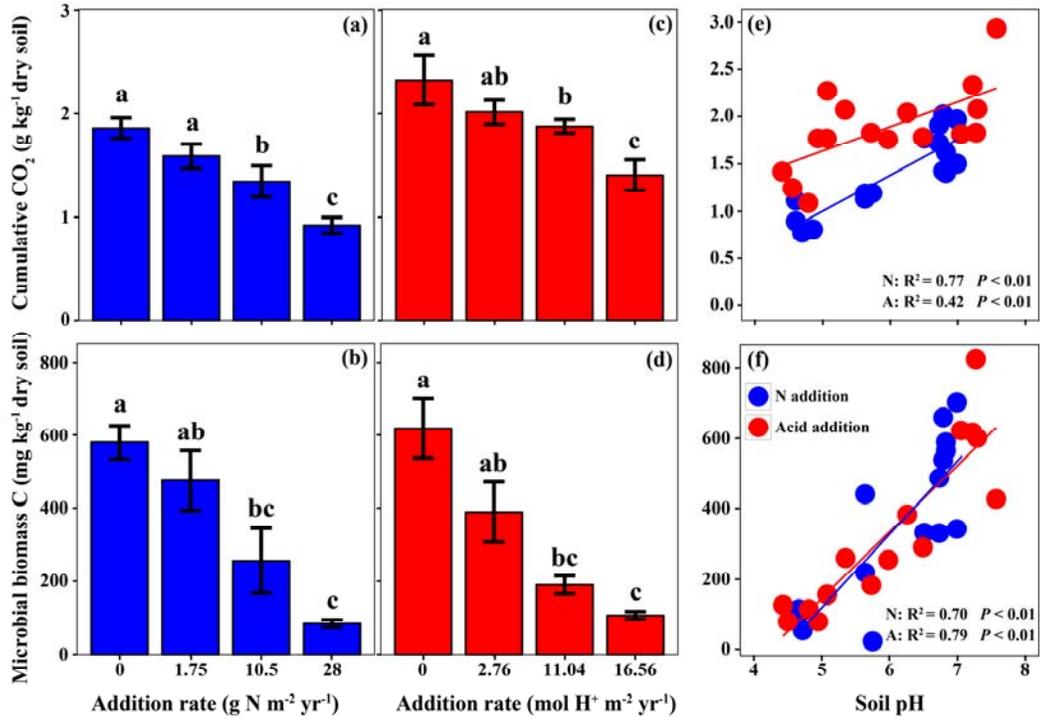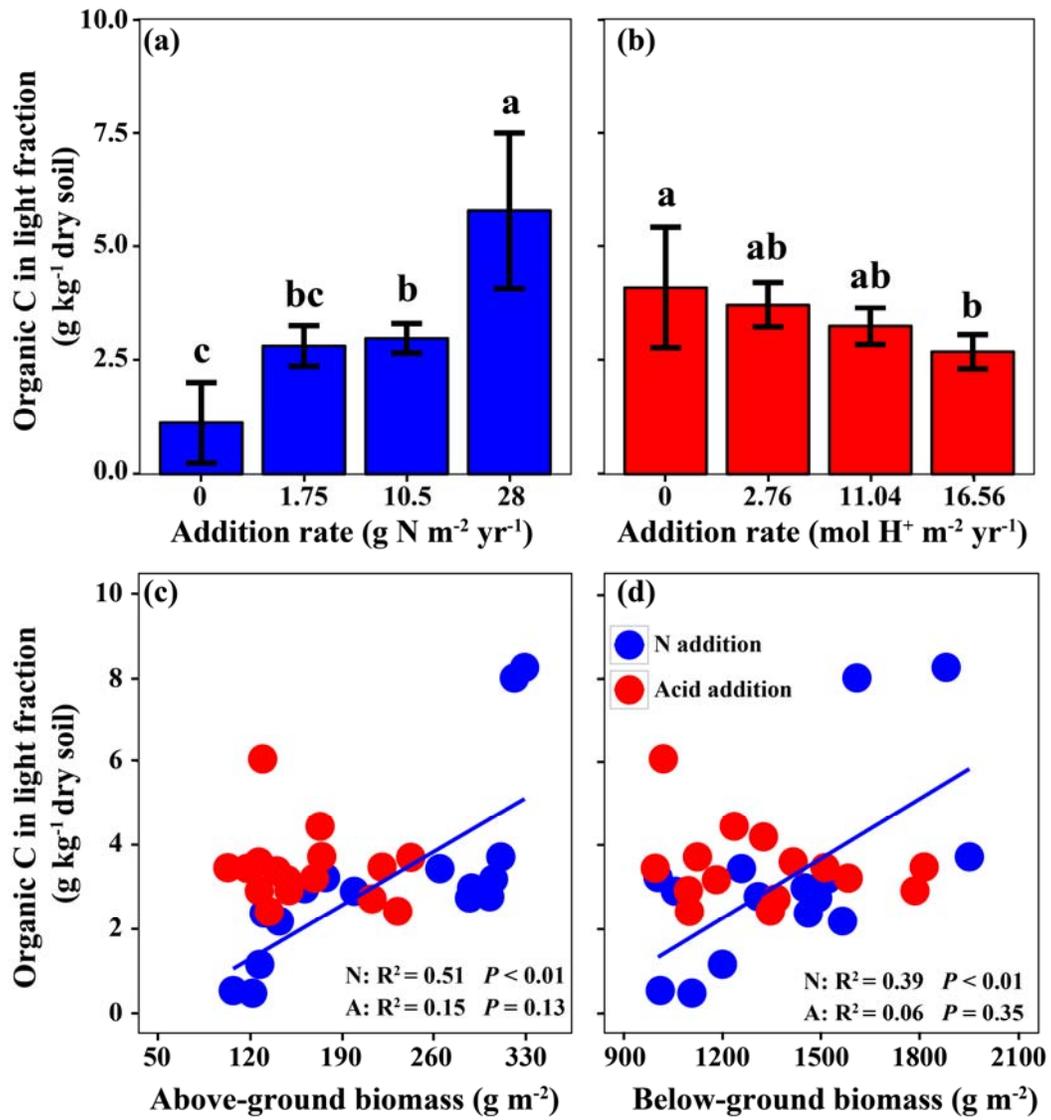
Figure 1

Figure 2

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