



Effects of micronutrient fertilization on soil carbon pools and microbial community functioning

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ABSTRACT

Fertilization with macronutrients, e.g., nitrogen (N) and phosphorus (P), has wide-ranging and well-characterized effects on terrestrial ecosystem functions. In contrast, influences of other elements, e.g., sodium (Na), calcium (Ca), and potassium (K), hereafter, ‘micronutrients’, are poorly understood. To determine effects of macronutrients, micronutrients, and their interactions on soil carbon (C) cycling, we conducted a full factorial experiment where treatments consisted of fertilizing experimental plots with Na, Ca, and K (each manipulated individually) and macronutrients (N + P added together). Each year of the experiment we measured above-ground plant biomass and sampled soils to quantify mineralizable C pools and active microbial biomass. To further explore micronutrient effects on microbial-mediated soil C cycling, in the final year of the experiment we assessed microbial C cycle functions using a catabolic profiling technique and quantified multiple additional soil C pools: particulate (POM-C), mineral-associated (MAOM-C), and total (TC). Macronutrients (N + P) affected nearly all measured variables – when N + P was added, active microbial biomass increased, catabolic profiles were altered, and mineralizable C, POM-C, and TC pools all increased. Micronutrients also had effects on soil C cycling that were generally smaller than effects of macronutrients – Ca addition reduced mineralizable C pools relative to the other treatments and K addition increased POM-C and TC pools. Further, Ca + K addition altered microbial catabolic profiles, but only when N + P was also added. Our study reveals several influences of micronutrients on soil C cycling – specifically, that Ca and K may influence the size of soil C pools by influencing the C cycle functions of soil microbial communities.

1. Introduction

Soil contains the largest terrestrial stock of carbon (C) globally (IPCC, 2013). Accumulation of C in soil depends upon the relative rates of processes that add C to soil (e.g., photosynthetic activity of plants) and processes that remove C from soil (e.g., decomposition and respiration of organic matter by soil microorganisms). The processes that influence soil C balance are sensitive to multiple anthropogenic global change drivers (e.g., Jansson and Hofmøckel, 2020; Osburn et al., 2021; Schimel, 2018; Wieder et al., 2015), thus representing potentially important feedbacks among human activities, soil, and climate. One anthropogenic influence on the soil C cycle that has received considerable research attention is nutrient fertilization; for example, nitrogen (N) and/or phosphorus (P) additions have altered C cycling in many ecosystems (e.g., Averill and Waring, 2018; Bradford et al., 2008; Crowther et al.,

2019; Janssens et al., 2010; Ramirez et al., 2010a, 2010b). Though the effects of N and P are widely studied and well-characterized, other elements, e.g., calcium (Ca), sodium (Na), and potassium (K), may also influence soil C cycling. These elements may be important in soil ecosystems because each is added to soils as a result of specific land management practices. For example, soil Ca increases following liming (Kellaway et al., 2022), Na can increase in soils following application of road deicers (Craig and Zhu, 2018) or Na-rich irrigation water (Gao et al., 2021), and K is a common component of crop fertilizers. However, these elements (hereafter, ‘micronutrients’) have received far less attention and are poorly understood within the context of the soil C cycle.

Macronutrient (i.e., N and P) availability is widely acknowledged to constrain the cycling and storage of C in terrestrial ecosystems (Vitousek and Howarth, 1991). Influences of N and P on soil C cycling can be

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attributed to effects of these nutrients on plants and on soil microbial processes. Here, we primarily focus on effects of nutrient fertilization on soil microbial processes. For example, microbial activity often declines with long-term N fertilization, potentially associated with soil acidification following N addition (Averill and Waring, 2018; Janssens et al., 2010; Ramirez et al., 2010a). These declines in microbial decomposer activity (i.e., reduced C respired), in turn, likely account for the greater soil C that is often observed following N fertilization (Bradford et al., 2008; Lu et al., 2011; Yue et al., 2016; Zak et al., 2017). N effects on soil C cycling may also be related to microbial community composition, which often shifts following N fertilization (e.g., Fierer et al., 2012; Pan et al., 2014; Ramirez et al., 2010b; Widdig et al., 2020). P addition has also been observed to increase soil C pools (Crowther et al., 2019; Yuan et al., 2020), though in some cases P addition has promoted soil C loss (Bradford et al., 2008; Poeplau et al., 2016). Similar to N, effects of P addition on soil C is likely related to changes in microbial activity and community composition (Huang et al., 2016; Li et al., 2015; Liu et al., 2012; Turner and Joseph Wright, 2014; Widdig et al., 2020). Increases in soil C with N and P addition are also likely related to increases in terrestrial primary productivity, which is known to be co-limited by multiple nutrients, including N and P (Fay et al., 2015; Harpole et al., 2011).

Though N and P can exert independent influence on soil C cycling, soil C storage also exhibits multiple nutrient co-limitation, where fertilization with combinations of N, P, and potentially other elements are required to observe increased soil C stocks (Crowther et al., 2019; van Groenigen et al., 2006). In particular, micronutrients, i.e., elements required by organisms in trace amounts, may be important in regulating terrestrial C cycling. Similar to macronutrients, effects of micronutrients on soil C balance will depend on how C addition processes (e.g., primary productivity) and C loss processes (e.g., decomposition/respiration) respond to micronutrient additions. For example, previous studies found both soil C storage and primary productivity to be stimulated by macronutrients and a cocktail of 9 micronutrients (Crowther et al., 2019; Fay et al., 2015). Litter decomposition processes have also been shown to be stimulated by a micronutrient cocktail (Kaspari et al., 2008), though these results have been contradictory depending on the micronutrient. For instance, other studies have shown that some micronutrients (e.g., zinc) can stimulate decomposition, while others (e.g., magnesium) can inhibit it (Powers and Salute, 2011). In general, these studies have demonstrated the potential for soil C cycle processes to be co-limited by several micronutrients – however, the influences of individual micronutrients remain largely unknown.

Though studies considering individual micronutrient effects on terrestrial ecosystems are rare, some isolated examples do exist. For example, the micronutrient calcium (Ca) is important for plant physiology and may therefore limit primary productivity (Kirkby and Pilbeam, 1984). Ca has also been shown to influence microbial diversity and microbial mineralization of organic C (Feng et al., 2016) and Ca may also be important for mineral-associated stabilization of soil organic C (Rowley et al., 2018). Another micronutrient, Na, may also influence soil C cycling by stimulating plant productivity and invertebrate herbivore activity (Jennings, 1976; Kaspari et al., 2017; Krishnasamy et al., 2014; Prather et al., 2018) and potentially by inhibiting microbial activity (Rietz and Haynes, 2003). Potassium (K) can also be considered a micronutrient for some organisms and may exert influence on the soil C cycle – K has long been known to be essential for plant growth and has been shown to influence soil microbial communities in some studies (e.g., Pan et al., 2014). Overall, though these studies illustrate the potential for individual micronutrients to affect terrestrial ecosystem processes, the roles of these micronutrients in influencing the soil C cycle remain poorly understood.

The goal of this study was to investigate the roles of the micronutrients Ca, Na, and K on grassland soil C cycling, and to determine if any soil processes were co-limited by macronutrients and any of these micronutrients. We chose these specific micronutrients because they

have been shown by previous studies and by our own preliminary analyses to be important in structuring grassland communities (e.g., Kaspari et al., 2017; Prather et al., 2018), and because their concentrations in soils may be altered by land management practices globally. To accomplish our study goals, we assessed soil C cycling in experimental plots that were fertilized with Ca, Na, and/or K (each manipulated individually) both with and without the addition of the macronutrients N and P (added together). We predicted that short-term N + P addition would stimulate microbial activity and associated C cycling and storage rates. We also predicted that Ca and K would promote soil C accumulation through mineral-associated C stabilization and stimulation of primary productivity, respectively. Finally, we predicted that the soil C cycle would exhibit multiple nutrient co-limitation, i.e., cycling and storage of C would be highest under fertilization with combinations of macro- and multiple micronutrients.

2. Materials and methods

2.1. Experimental design and soil sampling

We conducted this experiment in a coastal tallgrass prairie ecosystem in Texas at the University of Houston's Coastal Center (UHCC; 29°23'26.96" N; 95°1'51.95" W). The site contains soils in the Lake Charles series, classified as Hapluderts with a clay texture. Treatments for the study consisted of fertilizing with macronutrients (N and P added together) and three micronutrients (Ca, K and Na, each manipulated individually). The initial motivation for this study was to investigate micronutrient limitations on invertebrate herbivore communities – Ca, K, and Na are considered micronutrients for herbivores and were correlated with invertebrate abundances in preliminary analyses. As per Prather et al. (2018), the experiment consisted of fertilized $30 \times 30 \text{ m}^2$ plots using a fully-crossed, factorial design: 2 macronutrient (N + P) levels (ambient vs. fertilized) \times 2 Ca levels (ambient vs. fertilized) \times 2 K levels (ambient vs. fertilized) \times 2 Na levels (ambient vs. fertilized), for a total of 16 treatments. Each treatment was replicated 8 times for a total of 128 plots across 8 blocks. Treatments were assigned randomly to plots within each block and each block contained a single replicate of each treatment. Fertilization treatments began in 2016. Hurricane Harvey occurred on-site in late summer of 2017 creating heavy rainfall, which led to flooding of the study site.

Macronutrient (N + P) fertilizer was a combination of granular monoammonium phosphate and urea (i.e., $\text{NH}_4\text{H}_2\text{PO}_4 + \text{N}_2\text{H}_4\text{CO}$). The micronutrients were added as granular calcium carbonate (CaCO_3) for Ca, granular potassium chloride (KCl) for K, and granular soda ash (Na_2CO_3) for Na. Fertilizer treatments were applied in late winter in 2016 and 2017 before the start of the growing season. The macronutrients (N + P) were added at a concentration of 10 g m^{-2} , an application rate commonly used in fertilization experiments (e.g. Nutrient Network: Borer et al., 2014). Treatments with micronutrient additions were targeted to increase micronutrient concentrations ~30 % above ambient levels in the top 10 cm of soil. Accordingly, fertilizer was added to create micronutrient concentrations of 46.5 g m^{-2} for Ca, 3.1 g m^{-2} for K, and 6.2 g m^{-2} for Na.

2.2. Soil pH, plant biomass, mineralizable C, and microbial activity

At the end of the growing season in the years 2015–2018, we collected three 0–10 cm depth soil cores within each plot using a standard steel soil auger 8 cm in diameter. The three cores from each plot were composited, sieved at 4 mm and homogenized. We then measured soil pH, soil mineralizable C, and microbial activity in all collected soils. Soil pH was determined in water (2:1 ratio of DI water:soil) using a benchtop pH meter (Mettler Toledo, Columbus, OH, USA). To measure soil labile C content (i.e., mineralizable C), we conducted a 30-day C mineralization incubation with each soil sample. Incubations consisted of 6 g dry weight equivalent of soil weighed into 50 mL conical tubes. On

a weekly basis, tubes were capped, flushed with CO₂-free air, allowed to incubate for 24 h, and then the headspace gas sampled for CO₂ content using an infrared gas analyzer (IRGA; Model LI-7000, Li-Cor Biosciences, Lincoln, Nebraska, USA). Incubations were maintained at 65 % of the water holding capacity of each soil to maximize microbial activity. Total mineralizable C was calculated by integrating under CO₂ evolution time series curves. To estimate active microbial biomass, we used a substrate-induced respiration (SIR) method, where 4 g soil were incubated with an autolyzed yeast substrate for 5 h and then CO₂ was measured using an IRGA as described above. We additionally used the C mineralization and SIR data to calculate qCO₂ (i.e., metabolic quotient), for each sample. qCO₂ is a metric of microbial efficiency and was calculated according to Wardle and Ghani (1995), i.e., the ratio of basal respiration to SIR. We also measured plant biomass annually from 2016 to 2018 by sampling aboveground plant material from five 0.25m² quadrats per plot and then drying and weighing the collected plant material.

2.3. Soil carbon pools and microbial community functioning

For samples collected in the last year of sampling (2018), we measured total soil C (TC), particulate organic matter C (POM) and mineral-associated organic matter C (MAOM). Of these two fractions, mineral-associated C pools are presumed to be primarily microbial-derived C with slower turnover rates, whereas POM pools are primarily plant-derived with faster turnover rates (Bradford et al., 2008). To determine MAOM and POM C pools, we used the size fractionation method described in Bradford et al. (2008). Briefly, duplicate soil samples (10 g of air-dry soil) from each plot were dispersed with NaHMP (30 mL sample⁻¹) via shaking (18 h) and then passed through a 53 μm sieve. Material <53 μm is considered MAOM and material >53 μm is considered POM. Both mineral and POM material were dried (105 °C), ball-milled to a fine powder, and percentage C determined using an ECS 4010 CHNS-O analyzer (Costech Analytical Technologies, Valencia, CA, USA).

To assess microbial community function, we measured catabolic responses to multiple classes of C compounds abundant in soil, including simple sugars (i.e., glucose), amino acids (i.e., glycine), and organic acids (i.e., oxalic acid). We added each substrate to 4 g soil using the optimal concentrations of each class of substrate determined by Degens and Harris (1997). Microbial catabolic responses to each substrate were determined by measuring CO₂ in the headspace of each tube after incubating for 5 h using the IRGA method described above.

2.4. Statistical analyses

All statistical analyses were performed in R (R Core Development Team, 2019) using the lme4 and vegan packages (Bates et al., 2019; Oksanen et al., 2019). For variables assessed over multiple years (i.e., plant biomass, soil pH, mineralizable C, SIR), we determined effects of fertilization treatments using linear mixed effects models ('lmer' function, lme4 package). Models contained 'year' and individual fertilizer treatments as fixed effects and 'plot' nested within 'block' as a random effect, which accounts for repeated measurements from the same plots over time. We assessed assumptions of normality of residuals using q-q plots and when deviations from normality were observed, we used generalized linear mixed models ('glmer' function with gamma distribution and log link function, lme4 package). For variables assessed at the end of the experiment (i.e., catabolic responses, soil C fractions), we used mixed effects models with fertilization treatments as fixed effects and 'block' as a random effect. To determine effects of fertilization treatments on multivariate microbial catabolic profiles, we used PERMANOVA with Euclidean distances ('adonis2' function, vegan package) and visualized catabolic profiles using principal components analysis ('princomp' function, vegan package). For all statistical analyses, $P < 0.05$ was considered significant while $P < 0.1$ was considered marginally significant.

3. Results

3.1. Plant biomass and soil pH

To assess effects of macro- and micronutrients on aboveground ecosystem processes and key soil properties, we measured plant biomass and soil pH in each year of the experiment. Macronutrient (i.e., N + P) additions significantly increased plant biomass by 21 % on average (Fig. S1). Plant biomass also significantly varied among years with a large decline in 2017, likely due to effects of Hurricane Harvey (Fig. S1). Micronutrients also influenced plant biomass, evidenced by a significant N + P × Year × Na × K interaction, where K and Na addition increased plant biomass in the presence of N + P addition, but only in 2016 (Fig. S1). Macro- and micronutrient additions also influenced soil pH. Specifically, Ca addition increased pH, particularly in 2017 when N + P was also added (Fig. S2). Without Ca addition, N + P addition decreased soil pH (Fig. S2).

3.2. Mineralizable C and microbial activity

To quantify mineralizable C pools in soil, we measured cumulative C mineralization in all collected soils over 30 days. Soil mineralizable C generally increased in all fertilization treatments over time after treatments began in 2016 (Fig. 1A). Macronutrients had the largest effect on mineralizable C: N + P addition significantly increased mineralizable C by 17 % on average (Fig. 1A). Ca addition also influenced mineralizable C; on average, Ca addition decreased mineralizable C by 5 % relative to other treatments, though the effect was only marginally significant (Fig. 1B). Only N + P influenced active microbial biomass (i.e., SIR), and the N + P effect varied among years (Fig. 2). Specifically, N + P addition increased SIR, but only in 2016, where N + P plots had 16 % higher SIR than plots not receiving N + P (Fig. 2). Microbial efficiency (i.e., qCO₂) also varied among years and among fertilization treatments (Fig. S3). In particular, we identified a significant Year × N + P interaction, where N + P reduced qCO₂ (i.e., increased microbial efficiency) in 2016 and 2017 (Fig. S3).

3.3. Soil C fractions

To quantify effects of macro- and micronutrients on fast- and slow-turnover soil C pools, we quantified particulate (POM), mineral (MAOM), and total (TC) C pools in the last year of sampling (2018). Both macro- and micronutrients significantly influenced soil POM-C pools, where POM-C was 12 % higher when N + P was added and 11 % higher when K was added (Fig. 3A). These increases in POM-C led to increases in total C, where TC was 7 % higher with N + P addition and 5 % higher with K addition, though the K effect was only marginally significant (Fig. 3C). None of the fertilization treatments significantly affected MAOM-C (Fig. 3B).

3.4. Microbial catabolic profiles

To assess the effects of macro- and micronutrients on microbial function, we measured microbial catabolic responses to several C substrates in the last year of soil sampling (2018). Multivariate catabolic profiles were significantly affected by N + P addition (Fig. 4). This N + P effect was driven by generally greater microbial responses to all substrates, where responses to glucose, glycine, and oxalic acid were 20 %, 17 %, and 24 % higher, respectively, when N + P was added (Fig. S4). Catabolic profiles were also influenced by micronutrients, evidenced by a significant N + P × Ca × K interaction, where Ca + K addition influenced catabolic profiles but only when N + P was also added (Fig. 4). The N + P × Ca × K interaction is due to Ca + K addition amplifying the N + P effect on oxalic acid responses (Fig. S4C) but dampening the N + P effect on glucose responses (Fig. S4B). Ca also exhibited an independent significant effect on glucose metabolism and a marginally significant

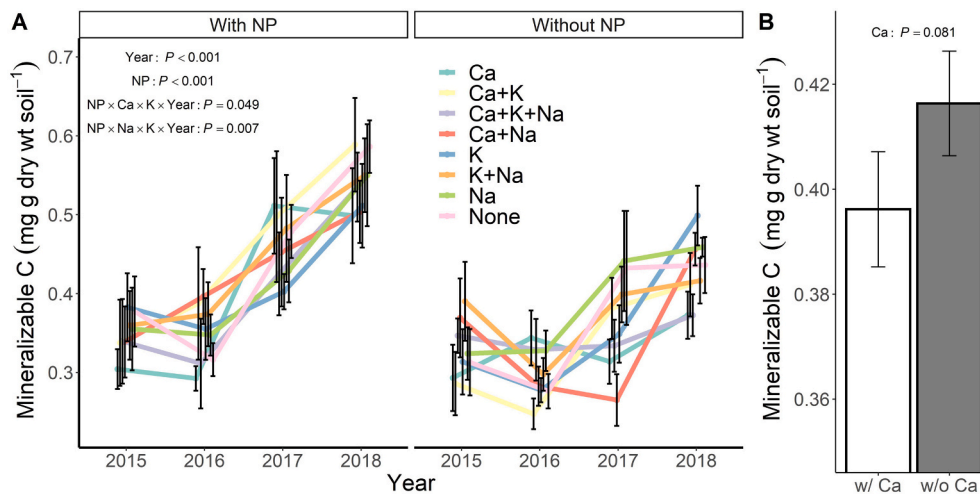


Fig. 1. Mineralizable C among all macronutrient (N + P) and micronutrient fertilization treatments across sampling years (A) and effects of Ca addition averaged across all other treatments and years (B). Values shown are treatment means, while error bars represent standard errors of the mean. *P* values are from mixed models with ‘year’ and fertilization treatments as fixed effects and ‘plot’ nested within ‘block’ as a random effect. On (A), only *P* values < 0.05 are shown.

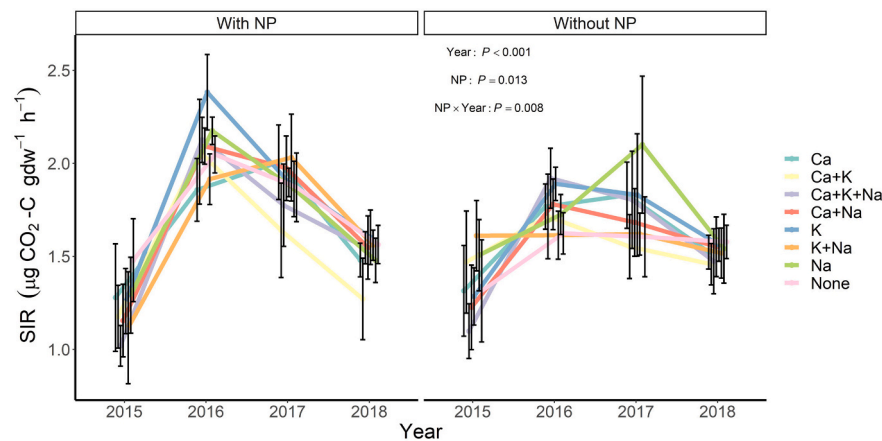


Fig. 2. Effects of micronutrients (with and without N + P addition) on active microbial biomass (SIR) across sampling years. Values shown are treatment means, while error bars represent standard errors of the mean. *P* values are from mixed models with ‘year’ and fertilization treatments as fixed effects and ‘plot’ nested within ‘block’ as a random effect. Only *P* values < 0.05 are shown.

effect on glycine metabolism, where soils with Ca addition had 8 % lower catabolic responses to both glucose and glycine (Fig. S4A, B).

4. Discussion

Our study reveals important influences of both macro- and micro-nutrients on soil C pools and microbial C cycle functioning. In general, macronutrients (N + P) had larger and more consistent effects on the soil C cycle variables we measured. However, individual micronutrients also had significant influences, including reduced mineralizable C pools, increased POM-C and total C pools, and altered microbial catabolic functioning. Further, in some cases the effects of micronutrients were dependent upon the presence of macronutrient additions, indicating that some soil C cycle processes are co-limited by several macro- and micronutrients. In general, this study shows that elements such as Ca and K represent underappreciated but important mediators of soil C cycling that are worthy of further investigation.

Similar to many previous studies, we found that macronutrient (N + P) additions resulted in larger soil C pools (e.g., Crowther et al., 2019; Lu et al., 2011; Yuan et al., 2020; Zak et al., 2017). Specifically, mineralizable C, POM-C, and total C all increased with N + P addition. Many prior studies have attributed increased soil C with N addition to reduced

microbial activity due to soil acidification (e.g., Averill and Waring, 2018). We also observed reduced soil pH with N + P addition. However, in contrast with previous studies, we observed greater soil C despite higher microbial activity (i.e., higher SIR) as well as greater catabolic responses to several C substrates when N + P was added. In our experiment, increased soil C with N + P addition may be partly attributed to greater plant-C inputs from litterfall and/or root exudates resulting from increased primary productivity. Indeed, plant biomass was significantly higher in our experimental plots where N + P was added. Additions of N + P may have also alleviated microbial nutrient limitation and therefore reduced microbial ‘mining’ of nutrients from soil organic C (Craine et al., 2007), thereby reducing respiration of C from organic matter. We also observed greater microbial efficiency with N + P addition, which may have contributed to increased soil C pools via increased input of microbial residues following biomass turnover (Cotrufo et al., 2013). Regardless, our results demonstrate that mechanisms other than reduced microbial decomposer activity may be responsible for increases in soil C following macronutrient fertilization.

In addition to effects of macronutrients, we observed significant influences of calcium (Ca) on soil C cycling. Specifically, we observed reductions in mineralizable C pools when Ca was added relative to the other fertilization treatments. In contrast to macronutrients, Ca addition

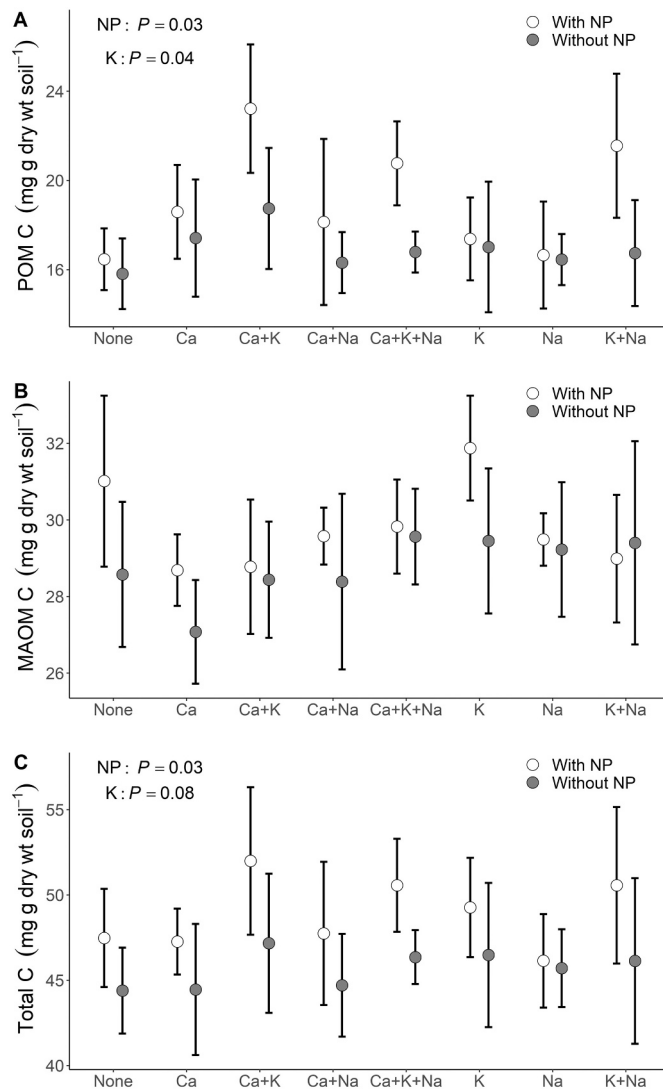


Fig. 3. Effects of micronutrients (with and without N + P addition) on soil POM-C (A), MAOM-C (B), and Total C (C) in the last year of sampling (2018). Values displayed are means, while error bars represent one standard error of the mean. P values are from mixed models with the fertilization treatments as fixed effects and ‘block’ as a random effect. Only P values < 0.1 are shown.

did not influence plant biomass, and therefore the effects of Ca on soil C cycling we observed are likely attributed to effects of Ca on microbial communities and mineral-organic C interactions rather than effects on primary productivity. For example, the reduction in mineralizable C with Ca addition we observed may be due to stabilization of soil organic C by formation of inner- and outer-sphere bridges by Ca^{2+} ions (Rowley et al., 2018), thereby reducing C available for microbial degradation. The effects of Ca on soil C cycling may also be partially attributed to effects of Ca on microbial community abundance and/or composition, as has been observed in other studies (Feng et al., 2016). This possibility is supported by our observation that Ca addition increased soil pH, which is known to be a dominant driver of microbial community composition in soils (e.g., Lauber et al., 2009).

We also observed effects of potassium (K) on soil C cycling. Specifically, fertilization with K increased both POM-C and total C pools. These increases in soil C are likely attributed in part to increases in primary productivity following K addition. Indeed, K has long been recognized as an important plant nutrient and K addition promoted increased aboveground plant biomass in our experiment. This greater plant productivity likely increased rates of root, leaf and/or root exudate material entering

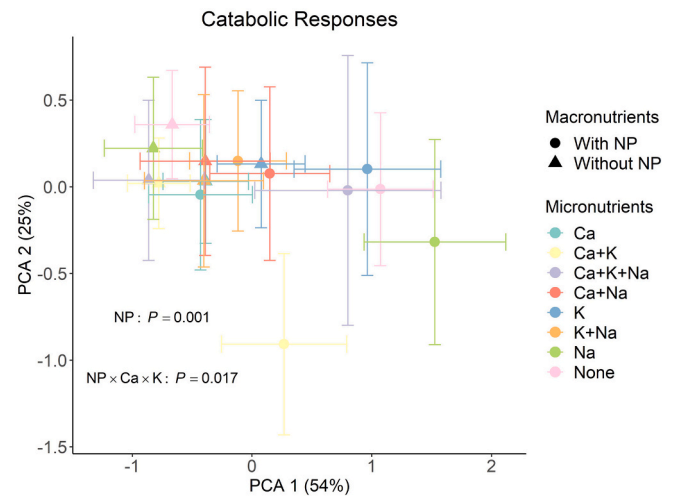


Fig. 4. Principal components analysis showing effects of micronutrients on microbial catabolic profiles in the last year of sampling (2018). Values displayed are centroids for each treatment while error bars represent standard errors. P values shown are from PERMANOVA with Euclidean distances. Only P values < 0.05 are shown.

soil pools, thereby contributing to the greater POM-C and total C pools we observed. The K effects on soil C may also be related to effects on microbial functioning, as K addition altered microbial catabolic profiles, though the effect was only evident when N + P and Ca were also added. Regardless, our results reveal K to be an important regulator of soil C cycling, both independently and in combination with macronutrients and other micronutrients.

Previous studies, including prior work from these same experimental plots, have found sodium (Na) and macronutrients (N + P) to co-limit insect herbivore abundance and diversity (Kaspari et al., 2017; Prather et al., 2018). Similarly, our study revealed aboveground plant biomass to be co-limited by Na and other nutrients (specifically, K and N + P). These influences of Na on plant productivity and herbivores suggest that Na may be important in regulating terrestrial C cycling. However, our results did not show any effects of Na on any soil C pools. It is possible that effects of Na on soil C pools in our experiment were small in magnitude and difficult to detect or may only manifest over longer time scales. Regardless, our study suggests that other micronutrient elements (e.g., K, Ca) are likely to be more important than Na in regulating the cycling and storage of C in grassland soils.

Importantly, it should be noted that our study was relatively short in duration and only considered effects of a select few elements indicated by preliminary analyses to be important in grassland ecosystems. However, many other micronutrient elements are required by organisms, e.g., zinc (Zn), cobalt (Co), magnesium (Mg), and iron (Fe), to name only a few (Wackett et al., 2004). These elements may have important influences on primary production, microbial community composition and function, and mineral-organic C interactions (e.g., Lalonde et al., 2012) and should be considered in future studies. Future studies should also investigate effects of micronutrient fertilization across multiple seasons and in deeper soil layers, as effects of these elements may be temporally and spatially variable. Finer classification of soil C pools into microaggregate and macroaggregate classes may also reveal interesting insights. Despite these limitations, however, our study shows that elements such as K and Ca can have important influences on soil C cycling and therefore these and other micronutrient elements should be comprehensively assessed within the context of soil ecosystem functioning.

5. Conclusions

Overall, our results largely support our original hypotheses: we observed increases in soil C with N + P addition as well as influences of K and Ca on soil C pools, which appeared to be linked to increases in primary productivity and mineral stabilization of organic C, respectively. We also found some evidence of multiple element co-limitation of the soil C cycle – plant biomass and microbial catabolic profiles were most significantly altered by combinations of macronutrients and multiple micronutrients. However, the soil C pools we quantified appeared to be influenced independently by individual fertilization treatments – mineralizable C was influenced independently by N + P and Ca, while POM-C and total C were influenced independently by N + P and K. Therefore, our results suggest that some soil C cycle components may not exhibit multiple element co-limitation. In addition, it should be noted that we observed no effects of our fertilization treatments on MAOM-C pools. This is important because MAOM-C is thought to be a slower turnover pool where soil C is stabilized over long time scales in grasslands (Bradford et al., 2008; Cotrufo et al., 2019). It is possible that changes in MAOM-C would manifest if micronutrient additions took place over longer durations. Alternatively, it is possible that MAOM-C pools in the clay-rich vertisols of this grassland ecosystem are saturated, and therefore MAOM-C would not exhibit changes. Regardless, our study reveals important influences of micronutrient elements on soil microbial communities and soil C pools, with implications for the cycling and storage of C across terrestrial ecosystems globally.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2022.104664>.

References

- Averill, C., Waring, B., 2018. Nitrogen limitation of decomposition and decay: how can it occur? *Glob. Chang. Biol.* 24, 1417–1427. <https://doi.org/10.1111/gcb.13980>.
- Bates, D., Maechler, M., Bolker, B., Runkler, S., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Scheipl, F., Grothendieck, G., Green, P., Fox, J., 2019. lme4: Linear Mixed-effects Models Using “Eigen” and S4. <https://doi.org/10.18637/journal.lme4>.
- Borer, E.T., Seabloom, E.W., Gruner, D.S., Harpole, W.S., Hillebrand, H., Lind, E.M., Adler, P.B., Alberti, J., Anderson, T.M., Bakker, J.D., Biederman, L., Blumenthal, D., Brown, C.S., Brudvig, L.A., Buckley, Y.M., Cadotte, M., Chu, C., Cleland, E.E., Crawley, M.J., Daleo, P., Damschen, E.I., Davies, K.F., DeCrappeo, N.M., Du, G., Firn, J., Hautier, Y., Heckman, R.W., Hector, A., HilleRisLambers, J., Iribarne, O., Klein, J.A., Knops, J.M.H., La Pierre, K.J., Leakey, A.D.B., Li, W., MacDougall, A.S., McCulley, R.L., Melbourne, B.A., Mitchell, C.E., Moore, J.L., Mortensen, B., O'Halloran, L.R., Orrock, J.L., Pascual, J., Prober, S.M., Pyke, D.A., Risch, A.C., Schuetz, M., Smith, M.D., Stevens, C.J., Sullivan, L.L., Williams, R.J., Wragg, P.D., Wright, J.P., Yang, L.H., 2014. Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* 508, 517–520. <https://doi.org/10.1038/nature13144>.
- Bradford, M.A., Fierer, N., Reynolds, J.F., 2008. Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. *Funct. Ecol.* 22, 964–974. <https://doi.org/10.1111/j.1365-2435.2008.01404.x>.

- Cotrufo, M.F., Ranalli, M.G., Haddix, M.L., Six, J., Lugato, E., 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. *Nat. Geosci.* 12, 989–994. <https://doi.org/10.1038/s41561-019-0484-6>.
- 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. <https://doi.org/10.1111/gcb.12113>.
- Craig, S., Zhu, W., 2018. Impacts of deicing salt and nitrogen addition on soil nitrogen and carbon cycling in a roadside ecosystem. *Water Air Soil Pollut.* 229, 187. <https://doi.org/10.1007/s11270-018-3838-6>.
- Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. *Ecology* 88, 2105–2113. <https://doi.org/10.1890/06-1847.1>.
- Crowther, T.W., Riggs, C., Lind, E.M., Borer, E.T., Seabloom, E.W., Hobbie, S.E., Wubs, J., Adler, P.B., Firn, J., Gherardi, L., Hagenah, N., Hofmockel, K.S., Knops, J.M.H., McCulley, R.L., MacDougall, A.S., Peri, P.L., Prober, S.M., Stevens, C.J., Routh, D., 2019. Sensitivity of global soil carbon stocks to combined nutrient enrichment. *Ecol. Lett.* 22, 936–945. <https://doi.org/10.1111/ele.13258>.
- Degens, B.P., Harris, J.A., 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. *Soil Biol. Biochem.* 29, 1309–1320. [https://doi.org/10.1016/S0038-0717\(97\)00076-X](https://doi.org/10.1016/S0038-0717(97)00076-X).
- Fay, P.A., Prober, S.M., Harpole, W.S., Knops, J.M.H., Bakker, J.D., Borer, E.T., Lind, E.M., MacDougall, A.S., Seabloom, E.W., Wragg, P.D., Adler, P.B., Blumenthal, D.M., Buckley, Y.M., Chu, C., Cleland, E.E., Collins, S.L., Davies, K.F., Du, G., Feng, X., Firn, J., Gruner, D.S., Hagenah, N., Hautier, Y., Heckman, R.W., Jin, V.L., Kirkman, K.P., Klein, J., Ladwig, L.M., Li, Q., McCulley, R.L., Melbourne, B.A., Mitchell, C.E., Moore, J.L., Morgan, J.W., Risch, A.C., Schütz, M., Stevens, C.J., Wedin, D.A., Yang, L.H., 2015. Grassland productivity limited by multiple nutrients. *Nat. Plants* 1, 1–5. <https://doi.org/10.1038/nplants.2015.80>.
- Feng, S., Huang, Y., Ge, Y., Su, Y., Xu, X., Wang, Y., He, X., 2016. Variations in the patterns of soil organic carbon mineralization and microbial communities in response to exogenous application of rice straw and calcium carbonate. *Sci. Total Environ.* 571, 615–623. <https://doi.org/10.1016/j.scitotenv.2016.07.029>.
- Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J.* 6, 1007–1017. <https://doi.org/10.1038/ismej.2011.159>.
- Gao, Y., Shao, G., Wu, S., Xiaojun, W., Lu, J., Cui, J., 2021. Changes in soil salinity under treated wastewater irrigation: a meta-analysis. *Agric. Water Manag.* 255, 106986. <https://doi.org/10.1016/j.agwat.2021.106986>.
- Harpole, W.S., Ngai, J.T., Cleland, E.E., Seabloom, E.W., Borer, E.T., Bracken, M.E.S., Elser, J.J., Gruner, D.S., Hillebrand, H., Shurin, J.B., Smith, J.E., 2011. Nutrient co-limitation of primary producer communities. *Ecol. Lett.* 14, 852–862. <https://doi.org/10.1111/j.1461-0248.2011.01651.x>.
- Huang, J., Hu, B., Qi, K., Chen, W., Pang, X., Bao, W., Tian, G., 2016. Effects of phosphorus addition on soil microbial biomass and community composition in a subalpine spruce plantation. *Eur. J. Soil Biol.* 72, 35–41. <https://doi.org/10.1016/j.ejsobi.2015.12.007>.
- IPCC, 2013. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Janssens, I.A., Dieleman, W., Luysaert, S., Subke, J.-a., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.-d., Tang, J., Law, B., 2010. Reduction of forest soil respiration in response to nitrogen deposition. *Nat. Geosci.* 3, 315–322. <https://doi.org/10.1038/ngeo844>.
- Jansson, J.K., Hofmockel, K.S., 2020. Soil microbiomes and climate change. *Nat. Rev. Microbiol.* 18, 35–46. <https://doi.org/10.1038/s41579-019-0265-7>.
- Jennings, B.D.H., 1976. The effects of sodium chloride on higher plants. *Biol. Rev.* 51, 453–486. <https://doi.org/10.1111/j.1469-185X.1976.tb01064.x>.
- Kaspari, M., Garcia, M.N., Harms, K.E., Santana, M., Wright, S.J., Yavitt, J.B., 2008. Multiple nutrients limit litterfall and decomposition in a tropical forest. *Ecol. Lett.* 11, 35–43. <https://doi.org/10.1111/j.1461-0248.2007.01124.x>.
- Kaspari, M., Roeder, K.A., Benson, B., Weiser, M.D., Sanders, N.J., 2017. Sodium co-limits and catalyzes macronutrients in a prairie food web. *Ecology* 98, 315–320. <https://doi.org/10.1002/ecy.1677>.
- Kellaway, E.J., Eimers, M.C., Watmough, S.A., 2022. Liming legacy effects associated with the world's largest soil liming and regreening program in Sudbury, Ontario, Canada. *Sci. Total Environ.* 805, 150321. <https://doi.org/10.1016/j.scitotenv.2021.150321>.
- Kirkby, E.A., Pilbeam, D.J., 1984. Calcium as a plant nutrient. *Plant Cell Environ.* 7, 397–405. <https://doi.org/10.1111/j.1365-3040.1984.tb01429.x>.
- Krishnasamy, K., Bell, R., Ma, Q., 2014. Wheat responses to sodium vary with potassium use efficiency of cultivars. *Front. Plant Sci.* 5, 631. <https://doi.org/10.3389/fpls.2014.00631>.
- Lalonde, K., Mucci, A., Ouellet, A., Gélinas, Y., 2012. Preservation of organic matter in sediments promoted by iron. *Nature* 483, 198–200. <https://doi.org/10.1038/nature10855>.
- Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.* 75, 5111–5120. <https://doi.org/10.1128/AEM.00335-09>.
- Li, J., Li, Z., Wang, F., Zou, B., Chen, Y., Zhao, J., Mo, Q., Li, Y., Li, X., Xia, H., 2015. Effects of nitrogen and phosphorus addition on soil microbial community in a

- secondary tropical forest of China. *Biol. Fertil. Soils* 51, 207–215. <https://doi.org/10.1007/s00374-014-0964-1>.
- Liu, L., Gundersen, P., Zhang, T., Mo, J., 2012. Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. *Soil Biol. Biochem.* 44, 31–38. <https://doi.org/10.1016/j.soilbio.2011.08.017>.
- Lu, M., Zhou, X., Luo, Y., Yang, Y., Fang, C., Chen, J., Li, B., 2011. Minor stimulation of soil carbon storage by nitrogen addition: a meta-analysis. *Agric. Ecosyst. Environ.* 140, 234–244. <https://doi.org/10.1016/j.agee.2010.12.010>.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. *vegan: Community Ecology Package*.
- Osburn, E.D., Simpson, J.S., Strahm, B.D., Barrett, J.E., 2021. Land use history mediates soil biogeochemical responses to drought in temperate forest ecosystems. *Ecosystems*. <https://doi.org/10.1007/s10021-021-00641-9>.
- Pan, Y., Cassman, N., de Hollander, M., Mendes, L.W., Korevaar, H., Geerts, R.H.E.M., van Veen, J.A., Kuramae, E.E., 2014. Impact of long-term N, P, K, and NPK fertilization on the composition and potential functions of the bacterial community in grassland soil. *FEMS Microbiol. Ecol.* 90, 195–205. <https://doi.org/10.1111/1574-6941.12384>.
- Poepflau, C., Herrmann, A.M., Kätterer, T., 2016. Opposing effects of nitrogen and phosphorus on soil microbial metabolism and the implications for soil carbon storage. *Soil Biol. Biochem.* 100, 83–91. <https://doi.org/10.1016/j.soilbio.2016.05.021>.
- Powers, J.S., Salute, S., 2011. Macro- and micronutrient effects on decomposition of leaf litter from two tropical tree species: inferences from a short-term laboratory incubation. *Plant Soil* 346, 245–257. <https://doi.org/10.1007/s11104-011-0815-x>.
- Prather, C.M., Laws, A.N., Cuellar, J.F., Reihart, R.W., Gawkins, K.M., Pennings, S.C., 2018. Seeking salt: herbivorous prairie insects can be co-limited by macronutrients and sodium. *Ecol. Lett.* 21, 1467–1476. <https://doi.org/10.1111/ele.13127>.
- R Core Development Team, 2019. *R: A Language And Environment for Statistical Computing*.
- Ramirez, K.S., Craine, J.M., Fierer, N., 2010a. Nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied. *Soil Biol. Biochem.* 42, 2336–2338. <https://doi.org/10.1016/j.soilbio.2010.08.032>.
- Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M.A., Fierer, N., 2010b. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology* 91, 3463–3470. <https://doi.org/10.1890/10-0426.1>.
- Rietz, D.N., Haynes, R.J., 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. *Soil Biol. Biochem.* 35, 845–854. [https://doi.org/10.1016/S0038-0717\(03\)00125-1](https://doi.org/10.1016/S0038-0717(03)00125-1).
- Rowley, M.C., Grand, S., Verrecchia, É.P., 2018. Calcium-mediated stabilisation of soil organic carbon. *Biogeochemistry* 137, 27–49. <https://doi.org/10.1007/s10533-017-0410-1>.
- Schimel, J.P., 2018. Life in dry soils: effects of drought on soil microbial communities and processes. *Annu. Rev. Ecol. Evol. Syst.* 49, 409–432. <https://doi.org/10.1146/annurev-ecolsys-110617-062614>.
- Turner, B.L., Joseph Wright, S., 2014. The response of microbial biomass and hydrolytic enzymes to a decade of nitrogen, phosphorus, and potassium addition in a lowland tropical rain forest. *Biogeochemistry* 117, 115–130. <https://doi.org/10.1007/s10533-013-9848-y>.
- van Groenigen, K.-J., Six, J., Hungate, B.A., de Graaff, M.-A., van Breemen, N., van Kessel, C., 2006. Element interactions limit soil carbon storage. *PNAS* 103, 6571–6574. <https://doi.org/10.1073/pnas.0509038103>.
- Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13, 87–115. <https://doi.org/10.1007/BF00002772>.
- Wackett, L.P., Dodge, A.G., Ellis, L.B.M., 2004. Microbial genomics and the periodic table. *Appl. Environ. Microbiol.* 70, 647–655. <https://doi.org/10.1128/AEM.70.2.647-655.2004>.
- Wardle, D.A., Ghani, A., 1995. A critique of the microbial metabolic quotient (qCO₂) as a bioindicator of disturbance and ecosystem development. *Soil Biol. Biochem.* 27, 1601–1610. [https://doi.org/10.1016/0038-0717\(95\)00093-T](https://doi.org/10.1016/0038-0717(95)00093-T).
- Widdig, M., Heintz-Buschart, A., Schleuss, P.-M., Guhr, A., Borer, E.T., Seabloom, E.W., Spohn, M., 2020. Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil. *Soil Biol. Biochem.* 151, 108041. <https://doi.org/10.1016/j.soilbio.2020.108041>.
- Wieder, W.R., Cleveland, C.C., Smith, W.K., Todd-Brown, K., 2015. Future productivity and carbon storage limited by terrestrial nutrient availability. *Nat. Geosci.* 8, 441–444. <https://doi.org/10.1038/ngeo2413>.
- Yuan, X., Qin, W., Xu, H., Zhang, Z., Zhou, H., Zhu, B., 2020. Sensitivity of soil carbon dynamics to nitrogen and phosphorus enrichment in an alpine meadow. *Soil Biol. Biochem.* 150, 107984. <https://doi.org/10.1016/j.soilbio.2020.107984>.
- 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. <https://doi.org/10.1038/srep19895>.
- Zak, D.R., Freedman, Z.B., Upchurch, R.A., Steffens, M., Kögel-Knabner, I., 2017. Anthropogenic N deposition increases soil organic matter accumulation without altering its biochemical composition. *Glob. Chang. Biol.* 23, 933–944. <https://doi.org/10.1111/gcb.13480>.