Running title: Anthropogenic change and net N mineralization

Global impacts of fertilization and herbivore removal on soil net nitrogen mineralization are modulated by local climate and soil properties

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Soil nitrogen (N) availability is critical for grassland functioning. However, human activities have increased the supply of biologically-limiting nutrients, and changed the density and identity of mammalian herbivores. These anthropogenic changes may alter net soil N mineralization (soil net N_{min}), i.e., the net balance between N mineralization and immobilization, which could severely impact grassland structure and functioning. Yet, to date, little is known about how these global change drivers individually, or collectively, affect soil net N_{min} across a wide range of grasslands that vary in soil and climatic properties. Here, we collected data from 22 grasslands on five continents, all part of a globally replicated experiment, to assess how fertilization and herbivore removal affected potential (laboratory-based) and realized (field-based) soil net N_{min}. Laboratory-based soil net N_{min} indicates the potential of grasslands to respond to global change, while realized soil net N_{min} shows how well grasslands can withstand global change under realistic field conditions.

Herbivore removal in the absence of fertilization did not alter potential and realized soil net N_{min}. However, herbivore removal in combination with fertilization, as well as fertilization alone, consistently increased potential soil net $N_{min.}$ Realized soil net N_{min}, in contrast, significantly decreased in fertilized plots where herbivores were removed. Site-specific soil and climatic properties strongly modulated treatment effects on potential and realized soil net N_{min}. Fertilization effects in potential soil net N_{min} were larger at sites with higher mean annual precipitation (MAP) and temperature of the wettest quarter (T.q.wet). Similarly, fertilization effects on realized soil net N_{min} were more negative at sites with lower MAP and higher T.q.wet. In summary, our findings show that anthropogenic nutrient enrichment, reductions in herbivores, and climatic conditions can negatively impact soil net N_{min} across global grasslands under realistic field conditions. This is important context-dependent knowledge for grassland management worldwide.

Keywords: anthropogenic change, grazers, nitrogen, phosphorus, potassium, potential and realized soil net nitrogen mineralization, precipitation, temperature, global grasslands, NutNet

Introduction

The availability of biologically limiting nutrients, such as nitrogen (N), phosphorus (P), and potassium (K), drives the productivity (Elser *et al.*, 2007; Harpole *et al.*, 2011; Fay *et al.*, 2015) and functioning of grassland ecosystems worldwide (Neff *et al.*, 2000). Soil N availability is largely determined by the breakdown and depolymerization of organic material to monomers and inorganic N for which plants and microbes compete (Schimel & Bennett, 2004; Butterbach-Bahl & Gundersen, 2011; Kuzyakov & Xu, 2013; Mooshammer *et al.*, 2014). The net balance between N mineralization and immobilization, further referred to as soil net N mineralization (soil net N_{min}), is largely controlled by soil physical properties (e.g., clay content, bulk density), soil carbon (C) and N content, the type and amount of above- and belowground organic matter inputs (e.g., plant production), plant and soil microbial activity (release of enzymes, nutrient uptake), and climatic factors (Giardina *et al.*, 2001; Schimel & Bennett, 2004; Booth *et al.*, 2005; Craine *et al.*, 2010; Dessureault-Rompré *et al.*, 2010; Conant *et al.*, 2011; Giese *et al.*, 2011; Risch *et al.*, 2019). Human activities that alter the biodiversity and structure of grassland plant communities worldwide also can have important direct and indirect consequences for the soil functioning, including soil net N_{min} (Rockström *et al.*, 2009; Steffen *et al.*, 2015). Two of the most pervasive human impacts threatening grassland ecosystems are increases in the supply of biologically limiting nutrients, e.g., through burning of fossil fuels or fertilization (Fowler *et al.*, 2013; Peñuelas *et al.*, 2013; Steffen *et al.*, 2015; Sardans *et al.*, 2017), and alterations in the density of native mammalian herbivores by over-exploitation or their replacement by livestock (Estes et al. 2011, Dirzo et al. 2014, Ripple et al. 2015, WWF 2018). However, we still do not know the relative contribution of climatic and edaphic factors versus anthropogenic drivers, such as increases in soil nutrient content and altered grazing, for the regulation of soil net N_{min} in global grasslands (e.g., Thébault *et al.*, 2014).

Grassland soil net N_{min} can be impacted by the presence and activity of herbivores through plant biomass consumption, trampling, burrowing, and deposition of urine and dung (e.g., Bakker *et al.*, 2004; Olofsson, 2009; Schrama *et al.*, 2013; Risch *et al.*, 2015; Zhou *et al.*, 2017). However, the response of a system to a change in grazing conditions depends on soil texture and water availability (Schrama *et al.*, 2013), grazing intensity (Zhou *et al.*, 2017), herbivore species identity (Risch *et al.*, 2015; Zhou *et al.*, 2017), and body-size (Bakker *et al.*, 2004; Risch *et al.*, 2015, 2018). Similarly, the impacts of anthropogenic nutrient inputs on grassland soil net N_{min} depend on plant and soil characteristics, as well as local climatic conditions (Mueller *et al.*, 2013; Changhui *et al.*, 2014; Ochoa-Hueso *et al.*, 2014; Wei *et al.*, 2017; Chen *et al.*, 2019a; Hicks *et al.*, 2019). Both herbivore removal and nutrient additions can have positive, negative, or neutral effects on soil net N_{min}, largely depending on site conditions (e.g., Bakker *et al.*, 2004; Changhui *et al.*, 2014; Risch *et al.*, 2015; Wei *et al.*, 2017). This strong context-dependency makes it difficult to estimate how increases in soil nutrient availability and shifts in the presence/absence of mammalian herbivores, individually and in combination, influence the ability of grassland soil communities to mineralize N from soil organic matter globally.

Reliably estimating soil net N_{min} is not straightforward, and methodological constraints can limit, or even misguide, our understanding of this key process under real-world, field conditions (Arnold *et al.*, 2008; Makarov *et al.*, 2017; Risch *et al.*, 2019; Pinto *et al.*, 2020). For example, measures of potential soil net N_{min} assessed in the laboratory may allow us to better understand the processes by which global change affect the overall magnitude of soil N availability across grasslands worldwide; i.e., they reflect the potential of grasslands to respond to global change (Risch *et al.*, 2019). Measures of realized soil net N_{min} obtained directly in the field, in contrast, may provide a more realistic indication of how grasslands will respond to fertilization and herbivore removal under heterogeneous environmental and climatic field conditions (Risch *et al.*, 2019). Moreover, these two distinct measures, potential and realized soil net N_{min}, only weakly correlate across grasslands worldwide (Risch *et al.*, 2019). Similarly, it is unclear whether knowledge about global change effects gained from laboratory assessments of soil net N_{min} may allow us to estimate what happens under real-world, field conditions. Thus, to understand and generalize how fertilization and herbivore removal affect soil net N_{min}, standardized globally replicated experiments are required. These experiments should span a wide range of environmental and climatic contexts, and measure both potential and realized soil net N_{min} simultaneously.

To fill this knowledge gap, we assessed how fertilization with limiting nutrients (N, P, K, plus nine essential macro- and micronutrients; NPK) and the removal of mammalian herbivores, individually (Fence) and in combination (NPK+Fence), affected potential and realized soil net N_{min} across 22 natural and semi-natural grasslands on five continents (Supplement Fig 1). Our sites spanned a comprehensive range of climatic and edaphic conditions found across the grassland biome (Fig 1; Supplementary Table 1 & 2). We focused on grasslands, because they cover 40-50% of the terrestrial landscape and provide many ecosystem functions and services. They are particularly important for forage production and C sequestration. Worldwide, grasslands store approximately 20-30% of the earth's terrestrial C, most of it in the soil (Schimel, 1995; White *et al.*, 2000). We assessed treatment differences in potential and realized soil net N_{min} using linear mixed-effects models (LMMs). We also analyzed how the treatments affected potential and realized soil net ammonification and net nitrification, which are the two main steps of soil net N_{min}. To gain a mechanistic system-level understanding of how fertilization and herbivore removal affect soil net N_{min}, we used structural equation modelling (SEM) to test a conceptual model that also considered the role of potentially modulating environmental and climate variables (Grace, 2006; Eisenhauer *et al.*, 2015).

Overall, we expected that our nutrient addition and grazing exclusion treatments, individually and in combination, should lead to higher soil nutrient availability and higher quality of plant litter returned to the soil (Coley *et al.*, 1985; Anderson *et al.*, 2018), which, in turn, should have a positive effect on both potential and realized soil net N_{min} across our global grasslands (Frank & Groffmann, 1998; Bakker *et al.*, 2004; Hobbie, 2015; Risch *et al.*, 2015; Ouyang *et al.*, 2018). However, we predicted that the magnitude in the response of potential soil net N_{min} to our treatments would be larger than the one of realized soil net N_{min} due to the optimal and standardized conditions during the laboratory incubations. Finally, we expected that treatment responses in both potential and realized soil net N_{min} would be modulated by soil properties and long-term climatic conditions, such as precipitation and temperature, because the structure and abundance of soil communities, and the processes they drive, are conditional on long-term water availability (Ochoa-Hueso *et al.*, 2018).

Methods

Study sites and experimental design

Our 22 sites are part of the Nutrient Network Global Research Cooperative (NutNet, https://nutnet.umn.edu/). Mean annual temperature across our 22 sites ranged from -4 to 22°C, mean annual precipitation from 252 to 1,592 mm, and elevations from 6 to 4,261 m above sea level (Fig 1, Supplementary Table 1). Soil organic C varied from 0.8 to 7.8%, soil total N from 0.1 to 0.6%, and the soil C:N ratio from 9.1 to 21.5. Soil clay content spanned from 3.0 to 35%, and soil pH from 3.4 to 7.6 (Supplementary Table 2). Thus, the sites covered a wide range of environments in which grasslands occur (Fig 1, Supplementary Table 1 & 2).

At each site, the effects of nutrient addition and herbivore removal treatments were tested via a randomized-block design (Borer *et al.*, 2014; Supplementary Fig 1A). Three replicate blocks with 10 treatment plots each were established at each site, with the exception of the site at bldr.us, where only two blocks were established (Supplementary Fig 1A). The 10 plots were randomly assigned to a nutrient or fencing treatment, but only a subset of four plots was used here, each with a different treatment (see below; Supplementary Fig 1A). All plots were 5 x 5 m and divided into four 2.5 x 2.5 m subplots (Supplementary Fig 1B). Each subplot was further divided into four 1 x 1 m square sampling plots, one of which was set aside for soil sampling (Borer *et al.*, 2014; Supplementary Fig 1B). Plots were separated by at least 1 m wide walkways.

In this study, we collected data from the following from four treatments: (i) untreated control plots (Control), (ii) herbivore removal plots (Fence), (iii) plots fertilized with N, P, K, plus nine essential macro and micronutrients (NPK), and (iv) plots with simultaneous fertilizer addition and herbivore removal (NPK+Fence; Supplementary Fig 1). Years of treatment differed among sites (2 – 9 years since start of treatment; Supplementary Table 1). For the nutrient additions, all sites applied 10 g N m^{-2} yr^{-1} as time-release urea; 10 g P m^{-2} yr^{-1} as triple-super phosphate; 10 g K m^{-2} yr^{-1} as potassium sulfate. A micro-nutrient mix (Fe, S, Mg, Mn, Cu, Zn, B, Mo, Ca) was applied at 100 g m^{-2} together with K in the first year of treatments but not thereafter to avoid toxicity.

The vertebrate removal treatment (Fence) was established by fencing two plots, one control and one NPK plot, within each of the blocks (Supplementary Fig 1). We designed the fences so that they would effectively exclude aboveground mammalian herbivores with a body mass of over 50 g (Borer *et al.*, 2014). At the majority of sites, the height of the fences was 180 cm, and the fence design included wire mesh (1 cm holes) on the first 90 cm along with a 30 cm outward-facing flange stapled to the ground to exclude burrowing animals; climbing and subterranean animals may potentially still access these plots (Borer *et al.*, 2014). For slight modifications in fence design see Supplementary Table 3. While most sites only had native herbivores, a few sites (4) were also grazed by domestic animals (Supplementary Table 1).

Potential and realized soil net N mineralization, ammonification, nitrification and other soil properties

Each site participating in the study received a package containing identical material from the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) to be used for sampling and on-site N incubations. For the field incubation, we followed the protocol by Risch *et al.* (2015, 2019). Briefly, we drove a 5 x 15 cm (diameter x depth) steel cylinder 13.5 cm deep into the soil after clipping the vegetation at randomized locations in each plot. The top 1.5 cm of the cylinder remained empty to capture incoming N from run-off or deposition with a polyester mesh bag (mesh-size 250 µm) filled with 13.2 ± 0.9 g of acidic and alkaline exchanger resin (1:1 mixture; ion-exchanger I KA/ion-exchanger III AA, Merck AG, Darmstadt). The bag was fixed in place with a metal Seeger ring (Bruetsch-Rüegger Holding, Urdorf, Switzerland). We then removed 1.5 cm soil at the bottom of the cylinder and placed another resin bag to capture N leached from the soil column. The exchange resin was saturated with H^+ and Cl^- prior to filling the bags by stirring the mixture in 1.2M HCl for 1 h and then rinsing it with demineralized water until the electrical conductivity of the water reached 5 µS/cm. The cylinders were then re-inserted into the cored hole, flush with the soil surface, and incubated for an average of 42 days (range 40 to 57days). The site coordinator chose the timing of incubation to start approximately six weeks prior to peak plant biomass production. All the incubations were completed between February 2015 and January 2016 accounting for differences in growing season between northern and southern hemispheres. At the end of the incubation, the cylinders were re-collected and immediately shipped to WSL in an insulated box together with cold packs to halt further mineralization. Gloves were worn at all times to avoid contamination of the samples. Upon arrival at WSL, we extracted the resin bags and a 20 g subsample of sieved soil (4 mm) separately in a 100 ml PE-bottle with 80 ml

1 M KCl for 1.5 h on an end-over-end shaker and filtered through ashless folded filter paper (DF 5895 150, ALBET LabScience). We measured NO_3^- (colorimetrically; Norman & Stucki, 1981) and NH_4^+concentrations (flow injection analysis; FIAS 300, Perkin Elmer) on these filtrates.

At the start of the field incubation, we additionally collected two soil cores of 5 x 12 cm (diameter x depth) in each sampling plot and composited them to measure potential soil net N_{min}, soil chemical and biological properties (see below). We then collected an additional sample (5 x 12 cm) to assess soil physical properties, which remained within the steel cylinder. Both ends were tightly closed with plastic caps. Then the core was gently packed to avoid further disturbance, and together with the composited soil samples, was shipped to the laboratory at WSL within a few days after collection.

From the composited samples, we extracted an equivalent of 20 g dry soil with KCl, as described above, and NO_3^- and NH_4^+concentrations were measured. Realized soil net N_{min} was then calculated as the difference between the inorganic N content of samples collected at the end of the incubation (plus N extracted from the bottom resin bag) and the N content at the beginning of the incubation and scaled to represent daily mineralization rates (mg N kg^{-1}soil d^{-1}; Risch *et al.*, 2015). Realized soil net N_{min} values represent an average period of 42 days prior to peak biomass, typically the highest period of biological activity, and not the entire year (Risch *et al.*, 2019).

A second subsample of the composited sample was used to determine potential soil net N_{min} in the laboratory (Risch *et al.*, 2019). Briefly, we weighed duplicate samples of soil equivalent to 8 g dry soil into 50-ml Falcon tubes. Soil moisture was brought to 60% of the field capacity of each plot, the Falcon tubes were tightly closed and then incubated at 20°C for 42 days in a dark room. Every week the Falcon tubes were opened and ventilated. At the end of the incubation, the soil samples were extracted the same way as described above and NO_3^- and NH_4^+ was determined. Potential soil net N_{min} was calculated as the difference between the N content before and after the incubation and scaled to represent daily values (mg N kg^{-1}soil d^{-1}). Using our NO_3^- and NH_4^+ measures we also calculated potential and realized soil net nitrification and soil net ammonification to be able to better understand how fertilization and herbivore removal affected potential and realized soil net N_{min}.

A third subsample of the composite soil sample was sieved (2 mm mesh) and microbial biomass (µg Cmic g^{-1} soil dry weight) was estimated by measuring the maximal respiratory response to the addition of glucose solution (4 mg glucose per g soil dry weight dissolved in distilled water; substrate-induced respiration method) on approximately 5.5 g of soil (Anderson & Domsch, 1978). The rest of the composited sample was dried at 65°C for 48 h and ground to pass a 2 mm mesh to assess a series of soil chemical properties (Risch *et al.*, 2019). We measured the percentage of clay as an indicator of soil texture (Gee & Bauder, 1986; Risch *et al.*, 2019).

Statistical analyses

Potential and realized soil net N_{min} were square root transformed to account for a highly skewed data distribution ($y_{t\,=}$ sign(y)*sqrt|y|; negative values in the data set impeded log transformation). To assess treatment effects on potential and realized soil net N_{min}, we used linear mixed effects models (LMMs) fitted by maximum likelihood using the lme function from the nlme package (version 3.131.1; Pinheiro *et al.*, 2016), R version 3.6.1; R Foundation for Statistical Computing. Treatment (Control, Fence, NPK, NPK+Fence) was a fixed factor, with site and block as random factors, where block was nested within site. We also tested for effects of time since start of treatments in preliminary analyses by adding total treatment years as an additional fixed factor. We did not find a significant effect of years of treatment, and thus dropped this variable from the models. The LMMs were corrected using varIdent if the homogeneity of variance criterion was not met (Pinheiro *et al.*, 2016). To visualize our results, we calculated treatment effects using Cohens’ d (Cohen, 1977; Koricheva *et al.*, 2013). Note that calculating response ratios (or log response ratios) is not possible with our data, as we have positive and negative values. We also fitted LMMs for potential and realized soil net ammonification and nitrification to gain more insight about how global change affects the processes underpinning potential and realized soil net N_{min}. We also sqrt-transformed (see above) these dependent variables. Treatment was included as a fixed factor with random factors as described above. In addition, we assessed how potential and realized soil net N_{min} were related to potential and realized soil net ammonification and nitrification, respectively. For this, we calculated site averages for each treatment separately. We then calculated LMMs, where potential and realized soil net N_{min} were the dependent variable, potential and realized soil net ammonification/nitrification the independent ones. Site was included as random factor.

Based on our previous work (Risch *et al.*, 2019) and the existing literature (Schimel & Bennett, 2004; Liu *et al.*, 2017), we developed a-priori causal conceptual models of relationships among treatments, environmental drivers, and potential and realized soil net N_{min} (Supplementary Fig 2) to test with structural equation modelling (SEM) using a *d-sep* approach (Shipley, 2009; Lefcheck, 2016). The variables included in the model were long-term climatic conditions, specifically site-level mean annual precipitation (MAP) and temperature of the wettest quarter (T.q.wet), plot-level soil texture (clay content) and soil microbial biomass. Mean annual precipitation and T.q.wet were obtained from WorldClim (Hijmans *et al.*, 2005) (http://www.worldclim.org/) and together with the experimental treatments were predicted to directly affect soil properties and soil net N_{min} (Supplementary Fig 2). Soil clay content was, in turn, predicted to affect microbial biomass and soil net N_{min}. Because we determined microbial biomass prior to incubating the samples in the laboratory or field, we assumed that the abundance of these microbes would be responsible for N process rates and not *vice versa* (Supplementary Fig 2). We tested our conceptual model (Supplementary Fig 2) using the piecewiseSEM package (version 2.0.2; Lefcheck, 2016) in R 3.4.0, in which a structured set of linear models are fitted individually. This approach allowed us to account for the nested experimental design, and overcome some of the limitations of standard structural equation models, such as small sample sizes (Shipley, 2009; Lefcheck, 2016). We used the lme function of the nlme package to model response variables, including site as a random factor. Good fit of the SEM was assumed when Fisher's C values were non-significant ($p > 0.05$). For all significant interactions with treatment detected in the SEMs, we calculated treatment effects, i.e. the differences in potential or realized soil net N_{min} between Control and treatments (Fence, NPK, NPK+Fence) and plotted these values against climate or soil variables. Finally, we fitted LMMs for the soil variables included in our SEMs (see below), with treatment as the fixed factor, and with site and block as random factors, where block was nested within site.

Results

Our treatments led to higher variability in both potential (Control: 0.465 ± 0.33 (mean ± SD); Fence: 0.53 ± 0.49; NPK: 0.689 ±0.63; NPK+Fence: 0.806 ± 0.56) and realized (Control: 0.336 ± 0.28; Fence: 0.345 ± 0.41; NPK: 0.317 ±0.75; NPK+Fence: 0.246 ± 0.77) soil net N_{min} across the 22 global grassland sites (see also Fig 2B,C, Supplementary Fig 3). As expected, our treatments increased potential soil net N_{min}: mineralization was higher in fertilized plots, both with (NPK: +34% on average across all sites) and without mammalian herbivores (NPK+Fence: +66%), but it did not respond to herbivore removal alone (Fence, Fig 2A,B, Supplementary Table 4). In contrast and counter to our hypotheses, realized soil net N_{min} was significantly lower in fertilized plots where herbivores were excluded compared to control plots (NPK+Fence: -42%, Fig 2A, Supplementary Table 4). However, herbivore removal (Fence) and fertilization (NPK) alone did not lead to any discernable difference in realized soil net N_{min} compared to the control plots (Fig 2A,C, Supplementary Table 4). Potential soil net ammonification only showed a negative but non-significant response to the NPK+Fence treatment (-29%), while realized soil net ammonification was significantly lower in both fertilized treatments (NPK: -44%, NPK+Fence: -61%) compared to the control (Fig 2D-F, Supplementary Table 4). In contrast, potential soil net nitrification was significantly higher in both fertilized plots compared to the control (NPK: +62%; NPK+Fence: +71%), while realized soil net nitrification remained unaffected (Fig 2G-J, Supplementary Table 4). In addition, potential soil net N_{min} was higher at sites with higher potential nitrification (Supplementary Figure 4A,B), and we found higher realized soil net N_{min} at sites with higher realized soil net ammonification and nitrification (Supplementary Figure 4C,D). Soil clay content and microbial biomass did not differ among our treatments (Supplementary Fig 5).

Our SEM explained 19% (marginal R^2) of the variability in potential soil net N_{min} across our grasslands and showed that potential soil net N_{min} increased directly in response to fertilization, regardless of the presence/absence of herbivores (Fig 3A), in line with the results of our LMM (Fig 2A). However, fertilization in combination with herbivore removal (NPK+Fence) had a larger positive effect on potential soil net N_{min} at sites with higher MAP (Fig 3A, Fig 4A). Similarly, we found larger increases in potential soil net N_{min} when nutrients were added at sites with higher temperature of the wettest quarter, regardless of herbivore presence/absence (NPK, NPK+Fence; Fig 3A, Fig 4B). Sites with higher MAP also had higher microbial biomass, which directly and positively affected potential soil net N_{min} (Fig 3A).

Our SEM for realized soil net N_{min} explained 33% (marginal R^2) of the variability in this measure across our grasslands (Fig 3B). Fertilization with herbivore removal directly and negatively affected realized soil net N_{min}, also in line with our LMM results (Fig 2B). However, we found that the negative joint effect of fertilization with herbivore removal (NPK+Fence) on realized soil net N_{min} was larger at sites with lower MAP and higher temperatures of the wettest quarter (Fig 4D,E). Moreover, the negative responses of realized soil net N_{min} to nutrient additions without herbivore removal (NPK) were conditional to sites with higher temperatures of the wettest quarter and more clay in the soil (Fig 3B, Fig 4D, E). Site-dependent decreases in realized soil net N_{min} in response to herbivore removal regardless of nutrient additions (Fence, NPK+Fence) were only evident at sites with greater microbial biomass (Fig 3B, Fig 4F). Sites with higher clay content and higher MAP had higher soil microbial biomass (Fig 3B).

Discussion

In this study, we did not detect any differences in potential or realized soil net N_{min} when herbivores were removed from global grasslands in the absence of fertilization. However, fertilization led to consistently higher potential soil net N_{min}, either individually (NPK), or when combined with herbivore removal (NPK+Fence). In contrast, and counter to our expectations, realized soil net N_{min}, was significantly lower compared to the control plots when we simultaneously added fertilizer and removed herbivores (NPK+Fence). This was surprising, as we expected increases in both potential and realized soil net N_{min} with our treatments, although with lower values for realized soil net N_{min}. We discuss potential reasons for these findings in detail below. Moreover, despite the overall patterns in treatment response in potential and realized soil net N_{min}, we found that site-specific differences in soil and climatic properties strongly influenced how fertilization and herbivore removal affected both potential and realized soil net N_{min}. This context-dependency together with the increased variabilities in both potential and realized soil net N_{min} caused by our treatments could be the underlying cause for the previous lack of consensus over the direction of grassland N mineralization responses to fertilizer additions and herbivore removal.

Herbivores alone do not affect potential and realized soil net N_{min} across global grasslands

The lack of a clear-cut response in soil net N_{min} to herbivore removal, in the absence of fertilization, is in line with a study conducted in the Swiss Alps, where the removal of large ungulates did not affect soil net N_{min} (Risch *et al.*, 2015). However, it contrasts with other findings where higher (Frank & Groffmann, 1998; Bakker *et al.*, 2004; Zhou *et al.*, 2017) or lower soil net N_{min} (Wang *et al.*, 2020) was detected in grazed compared to ungrazed grasslands. In our study, we only found a reduction in realized soil net N_{min} with herbivore removal at sites with higher microbial biomass; however, this context-dependent response was not strong enough to allow for a clear overall pattern to emerge across sites. Apart from microbial biomass, differences in population densities, stocking rates, or herbivore community composition that result in different grazing intensities may also contribute towards explaining our variable responses, but we lacked this information in our study. A recent meta-analysis, which included mixed data of both potential and realized soil net N_{min}, showed, however, that moderate grazing led to significantly higher soil net N_{min} compared to ungrazed plots across global grasslands, whereas light and heavy grazing may have no effect (Zhou *et al.*, 2017). Similarly, the removal of large ungulates alone did not affect realized soil net N_{min} in the Swiss Alps, but when all mammalian herbivores were excluded and only invertebrates were present, realized soil net N_{min} increased (Risch *et al.*, 2015). In a Dutch grassland, only the removal of cattle led to increases in realized soil net N_{min}, while the additional removal of rabbits and voles did not lead to further changes (Bakker *et al.*, 2004). These studies highlight the importance of considering the functional diversity of the excluded herbivores (Wang *et al.*, 2019). Finally, time since treatment implementation, i.e., establishing the fences, may potentially explain some variability in the response of grassland mineralization to herbivore removal (Frank & Groffmann, 1998; Bakker *et al.*, 2004; Risch *et al.*, 2015; Wang *et al.*, 2020), but we did not find any statistical evidence for this in our study.

Nutrient addition alone and with herbivore removal affects potential and realized soil net N_{min}

We found higher potential soil net N_{min} in both fertilized treatments (i.e., NPK, NPK+Fence) compared to the control plots. We are not aware of other studies that assessed how potential soil net N_{min} responded to NPK fertilizer additions, but N additions increased potential soil net N_{min} in a semi-arid grassland with loamy-sand soils (Chen *et al.*, 2019a). Our findings suggest that in our fertilized treatments, soil microbes, either generally or certain microbial groups specifically, consistently increased their activity under the standardized and optimized conditions in the laboratory, particularly for samples collected from sites with higher mean annual precipitation and higher temperature of the wettest quarter. This context-dependency on long-term climatic conditions may, in turn, be mediated by greater plant productivity and soil microbial biomass at wetter and warmer sites leading to a greater pool of readily mineralizable soil organic N. This finding is similar to what has been shown in the Mongolian steppe, where fertilization increased potential soil net N_{min} under both moderate and heavy grazing, but with additional irrigation, potential soil net N_{min} was only higher at the moderately grazed sites (Chen *et al.*, 2018).

In contrast, fertilization alone (NPK) did not alter realized soil net N_{min} across our global grasslands, which is similar to results reported from several site-specific fertilization experiments (Mueller *et al.*, 2013; Wei *et al.*, 2017). Fertilization in combination with herbivore removal (NPK+Fence) did, however, lead to a decrease in soil net N_{min}, which was unexpected, yet similar to findings from a Californian serpentine grassland study (Esch *et al.*, 2013). This decrease might be due to the strong context-dependency in the response of realized soil net N_{min} to our nutrient additions, with or without herbivores. Fertilization led to lower realized mineralization rates at sites with higher soil clay content (NPK) and higher temperature of the wettest quarter (NPK, NPK+Fence). The treatment interaction with soil clay content could potentially be attributed to the fact that our treatments disrupted organo-mineral interactions within the soil matrix (Zhao *et al.*, 2020). Moreover, given that higher soil clay content was generally associated with higher microbial biomass, our findings also suggest that microbial communities are downregulating the ‘mining’ for nutrients from soil organic matter and release less mineral nutrients when limiting nutrients are added (Dijkstra *et al.*, 2013). Decreases in the activity of nitrifying bacteria after grazing cessations were indeed documented in a mesocosm study (Le Roux *et al.*, 2007), which partially supports our findings, although this study did not include nutrient additions. A recent meta-analysis including field-based studies from different terrestrial biomes assessed how changes in enzyme activities were related to the application rates of N. Generally, at N application rates of 100 kg ha^{-1} y^{-1}, which are equivalent to our additions, enzyme activities were negatively affected by N additions (Jia *et al.*, 2020). Yet, four years of adding N and P fertilizer had a minor effect on enzyme activities in three different Chinese grassland systems (Chen *et al.*, 2019b). Similarly, fertilization and herbivore removal individually or in combination did not affect microbial extracellular enzyme activities in a Californian grassland (Esch *et al.*, 2013), further emphasizing the variability of results found across studies.

Differences in the response of potential and realized soil net N_{min} to nutrient additions and herbivore removal

In contrast to our expectations, our treatments only led to increases in soil net N_{min} in the laboratory under standardized and optimal conditions, but not in the field, where our treatments lead to a reduction in realized soil net N_{min}. This shows that laboratory measures, although useful to explore and understand soil processes under standardized conditions, do not allow us to anticipate what happens under ambient conditions in the field, as previously suggested by other studies (Arnold *et al.*, 2008; Risch *et al.*, 2019). Hence, to predict and estimate how global change drivers such as biodiversity loss, caused by fertilization and climate change, alter the rates of N mineralization in grassland ecosystems, it is important to measure soil N processes in the field and not in the laboratory.

Mechanistically, the differences in the response of potential and realized soil net N_{min} to our treatments may be attributed to a combination of sample preparation (mixing, sieving, removing roots) and a shift in the composition or activity of different microbial groups under optimal versus ambient conditions. In the field, dead roots remained in the incubated soil cores while the roots were removed for the laboratory incubations. Hence, more labile C was available in the field, which is known to increase N immobilization and decrease soil net N_{min} (Hook & Burke, 1995; Knops *et al.*, 2002). Similarly, soil preparation for determining potential soil net N_{min} makes formerly protected soil organic matter available and fine aggregates were shown to have much higher potential N mineralization than coarse aggregates (Bimüller *et al.*, 2016) or undisturbed soils (Hassink, 1992). In addition, microbial communities associated with different sized soil aggregates were shown to interact with NPK fertilizer (Liao *et al.*, 2018). Further, we found higher potential soil net nitrification with fertilization (regardless of presence or absence of herbivores), while there was only a weak effect of NPK+Fence on potential soil net ammonification. In contrast, realized soil net nitrification remained unaffected by our treatments. This indicates that nitrifiers likely were more active in the laboratory when limiting nutrients were added as indicated by much higher nitrification rates compared to the field under fertilized conditions. Hence, more NO_3-N was processed when fertilized and potential soil net N_{min} increased. In line with these findings, potential soil net nitrification was higher in NPK fertilized agricultural soil under maize compared to the control (Li *et al.*, 2019). The authors explained the enhanced potential net nitrification with a higher abundance of ammonia oxidizing bacteria and archaea (nitrifying microbes). Similarly, the negative response of realized soil net N_{min} to fertilization could be due to the lower activity of ammonifiers, as we found lower realized soil net ammonification when fertilizer was added (regardless of herbivore presence/absence), but no change in realized soil net nitrification. Consequently, less NH_4-N was released, which ultimately led to lower realized soil net N_{min}. Unfortunately, we were not able to find any studies that assessed how fertilization or grazing affects the relationship between the activities of nitrifiers/ammonifiers and net ammonification/nitrification, and how this would feed back to potential and realized soil net N_{min} to compare with our result. Hence, further studies should evaluate the role of different microbial taxa in the regulation of soil organic matter processing and nutrient cycling under different management regimes, which may allow for the identification of specific communities that function better under particular conditions.

Finally, across our grasslands, the response of both potential and realized soil net N_{min} to fertilization with herbivore removal (NPK+Fence), and potential soil net N_{min} to fertilization only (NPK), depended on the mean annual precipitation and temperature of the wettest quarter. Thus, expected alterations in global precipitation regimes (Fischer & Knutti, 2014) due to global climate change will likely have a strong impact on grassland soil net N_{min} in combination with different grassland management regimes, as shown by Chen *et al.* (2018). Similarly, N additions in combination with increases in soil water availability had more consistent positive effects on nitrogen-mineralizing enzyme activities than the two factors in isolation (Tian *et al.*, 2017).

In conclusion, our study provides strong evidence that human activities impact the capacity of grassland ecosystems to provide key ecosystem functions such as soil net N_{min}. We show that a nutrient-enriched, herbivore-impoverished, and climatically variable world will have negative consequences for the ability of soil communities to mineralize N under realistic field conditions. In the long-term, this might lead to a reduction of grasslands' functional ability to mineralize soil N, making them increasingly dependent on external inputs. Thus, our findings strongly support farmers and land managers advocating to move away from high input agriculture and promote a more sustainable management of grassland ecosystems and their soils. Moreover, our results show under which environmental conditions, fertilization and herbivore removal cause the strongest negative effects on soil nutrient cycling and may thus require particular attention.

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Figure captions

Fig 1. Geographic and climatic distribution of experimental sites. (A) Location of the 22 NutNet sites where the field experiment was conducted and soil samples were collected for laboratory analyses. (B) The 22 study sites represent a wide range of mean annual temperature (MAT) and mean annual precipitation (MAP) conditions. Our sites also cover a wide range of soil edaphic conditions as described in the main text and shown in Supplementary Table 2. Numbers refer to # in Supplementary Table 1 & 2.

Figure 2: Treatment effects on potential and realized soil net N mineralization across 22 grasslands globally. Potential and realized A-C) soil net N_{min}, D-F) soil net ammonification, G-J) soil net nitrification. Treatment effect size presented as Cohens' d in A, D and G. Significant treatment effects are indicated with *. Boxplots of raw data (B, C, E, F, H, J) show the median (50th percentile), 25th and 75th percentile of the data across sites. Individual measures are shown in the background. Control = control plots, Fence = herbivores removed, NPK = fertilized with N, P, K and micronutrients, NPK+Fence = fertilized with N, P, K and micronutrients and herbivores removed. Potential and realized soil net N_{min}, net ammonification and nitrification values were square-root transformed.

Figure 3: Influence of local environmental conditions on the response of potential and realized soil net N mineralization (soil net N_{min}) to herbivore presence/absence and nutrient additions across global grasslands. (A) Structural equation model diagram representing connections between climatic conditions and soil properties found to influence potential soil net N_{min}. (B) Structural equation model diagram representing connections between climatic conditions and soil properties found to influence realized soil net N_{min}. The width of the connections represents estimates of the standardized path coefficients, with solid lines representing a positive relationship and dashed lines a negative relationship. Interaction effects are depicted with arrows pointing to solid blue dots. Significant connections and R^2 are shown in black, non-significant ones in light-grey. †$p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. MAP = mean annual precipitation, T.q.wet = temperature of the wettest quarter, Treatments: Control = control plots, Fence = herbivores removed, NPK = fertilized with N, P, K and micronutrients, NPK+Fence = fertilized with N, P, K and micronutrients and herbivores removed, Clay content = soil clay content, Micr. Biom. = soil microbial biomass, total number of observations for potential soil net N_{min} = 244, total number of observations for realized soil net N_{min}. = 256, total number of sites for potential soil net N_{min} = 21, total number of sites for realized soil net N_{min} = 22. Potential and realized soil net N_{min} values are square-root transformed.

Figure 4: Treatment effects on soil net N_{min} in relation to site-specific differences in climate or soil properties for which interactions with treatment were found in the SEMs. A, B) Relationships for potential soil net N_{min}; C, D, E, F) Relationships for realized soil net N_{min}. Control = control plots, Fence = herbivores removed, NPK = fertilized with N, P, K and micronutrients, NPK+Fence = fertilized with N, P, K and micronutrients and herbivores removed. Potential and realized soil net N_{min} values are square-root transformed. Treatment effects were calculated as the difference between values on control and treatment (Fence, NPK, NPK+Fence) plots.

Figures

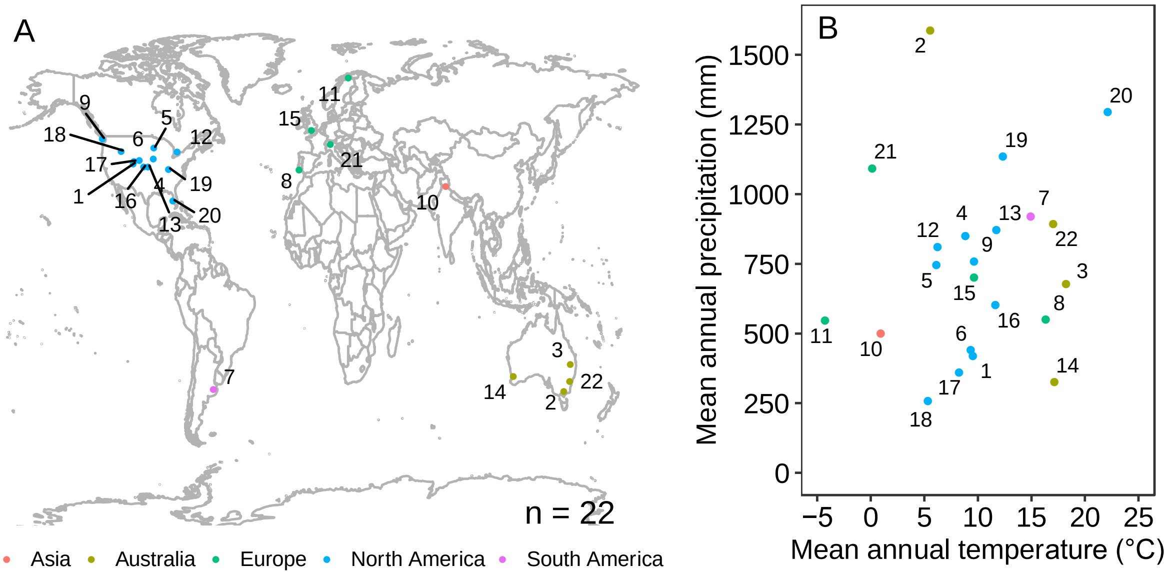


Figure 1

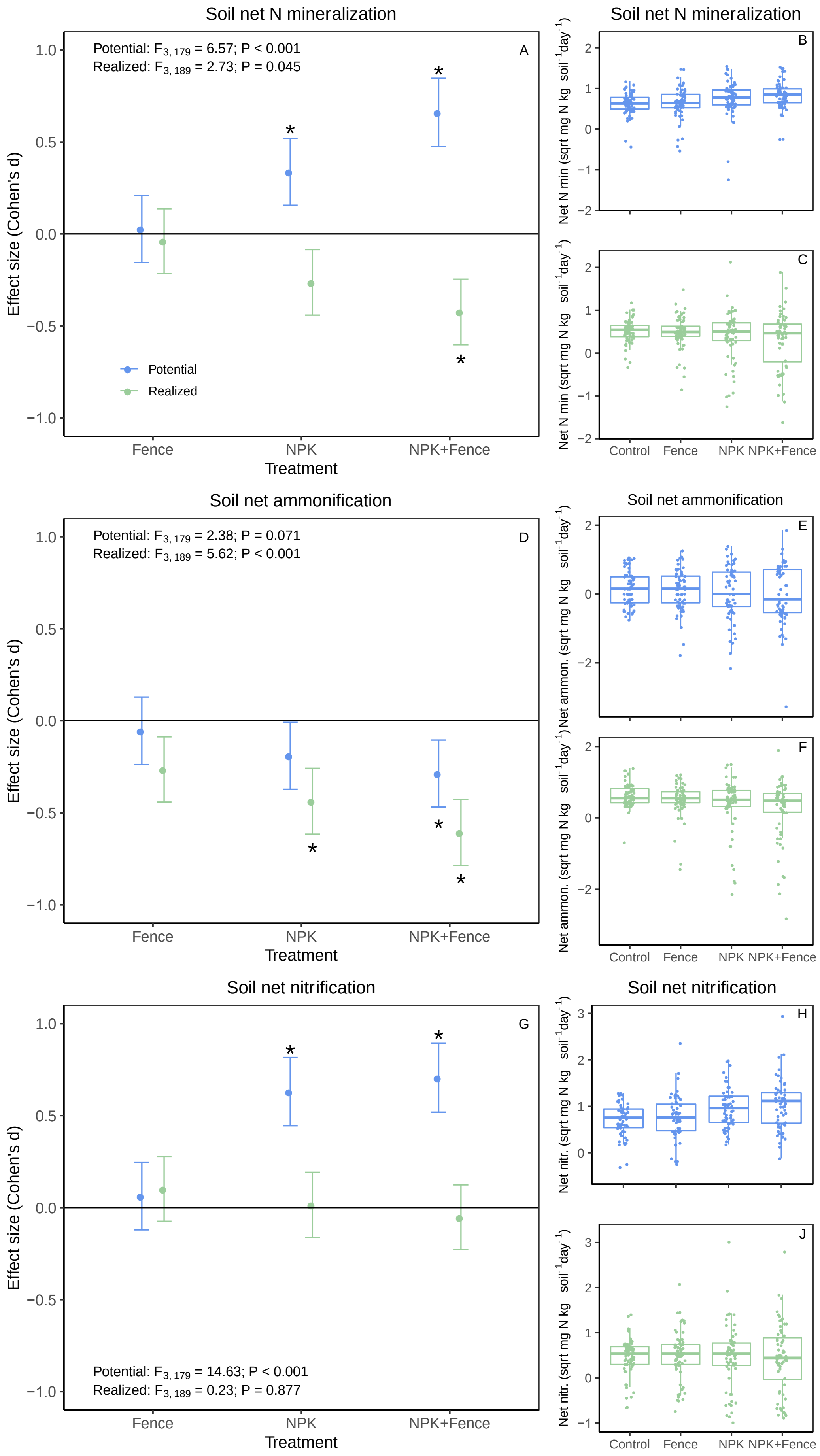

Soil net N mineralization
A
Potential: $F_{3,\,179}$ = 6.57; P < 0.001
Realized: $F_{3,\,189}$ = 2.73; P = 0.045
Effect size (Cohen's d)
1.0
0.5
0.0
−0.5
−1.0
Potential
Realized
*
Fence
NPK
NPK+Fence
Treatment
Soil net N mineralization
B
C
Net N min (sqrt mg N kg soil^{-1}day^{-1})
Control
Fence
NPK
NPK+Fence
Soil net ammonification
D
Potential: $F_{3,\,179}$ = 2.38; P = 0.071
Realized: $F_{3,\,189}$ = 5.62; P < 0.001
Soil net ammonification
E
F
Net ammon. (sqrt mg N kg soil^{-1}day^{-1})
Soil net nitrification
G
Potential: $F_{3,\,179}$ = 14.63; P < 0.001
Realized: $F_{3,\,189}$ = 0.23; P = 0.877
Soil net nitrification
H
J
Net nitr. (sqrt mg N kg soil^{-1}day^{-1})

Figure 2

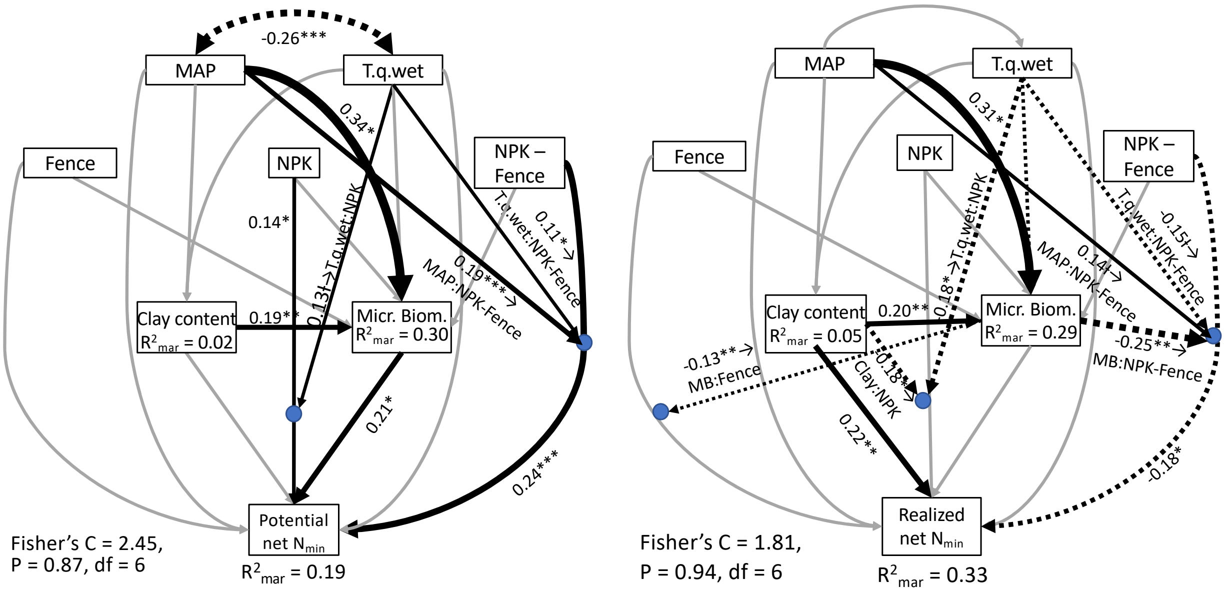

-0.26***
MAP
T.q.wet
Fence
NPK
NPK – Fence
0.34*
0.14*
0.13† → T.q.wet:NPK
0.11* → T.q.wet:NPK-Fence
0.19*** → MAP:NPK-Fence
Clay content R2mar = 0.02
0.19**
Micr. Biom. R2mar = 0.30
0.21*
0.24***
Potential net Nmin
R2mar = 0.19
Fisher's C = 2.45, P = 0.87, df = 6
MAP
T.q.wet
Fence
NPK
NPK – Fence
0.31*
-0.18* → T.q.wet:NPK
-0.15† → T.q.wet:NPK-Fence
0.14† → MAP:NPK-Fence
Clay content R2mar = 0.05
0.20**
Micr. Biom. R2mar = 0.29
-0.13** → MB:Fence
-0.18* → Clay:NPK
0.22**
-0.25** → MB:NPK-Fence
-0.18*
Realized net Nmin
R2mar = 0.33
Fisher's C = 1.81, P = 0.94, df = 6

Figure 3

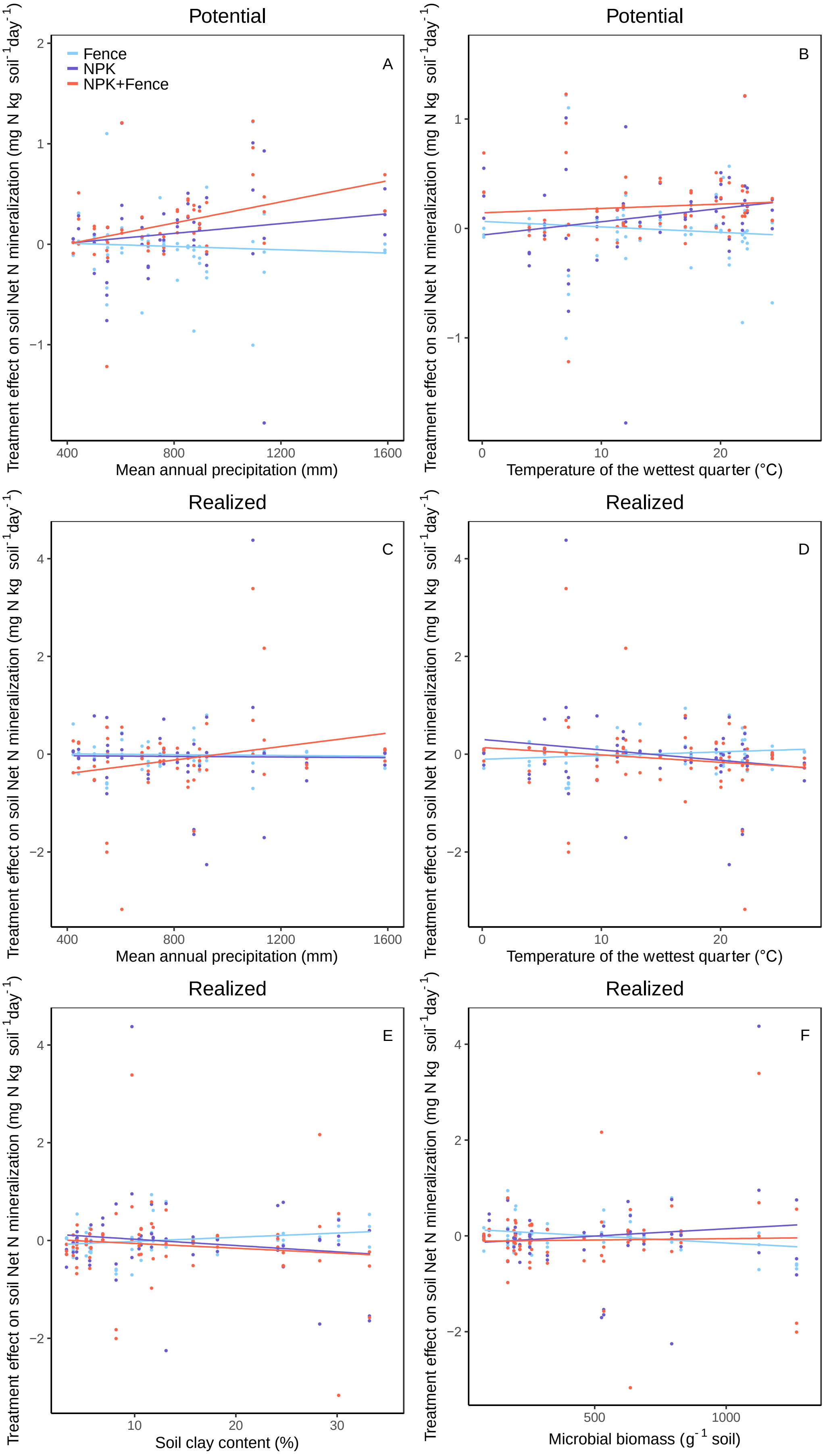
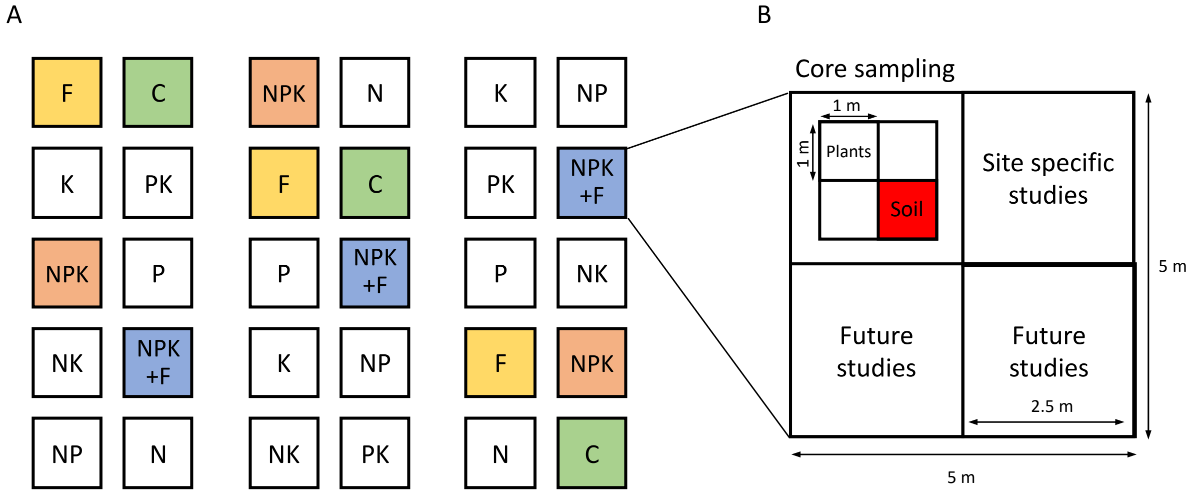
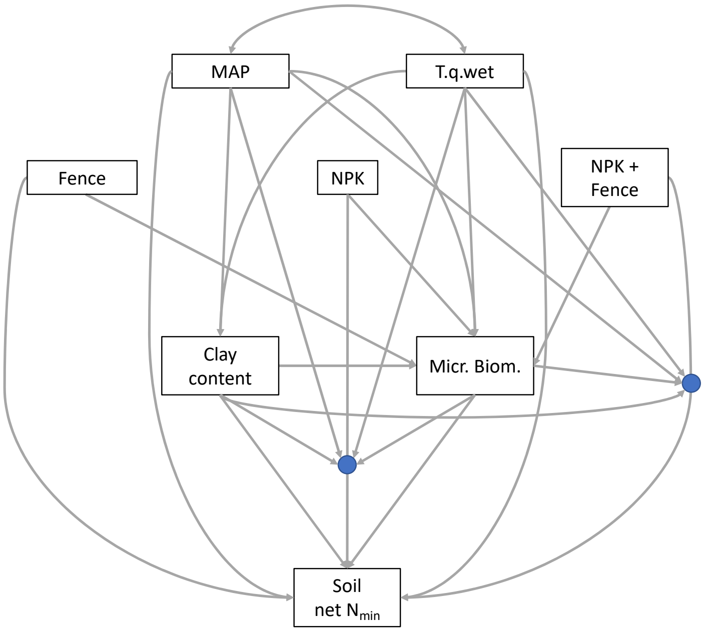

Potential
A
Fence
NPK
NPK+Fence
Treatment effect on soil Net N mineralization (mg N kg soil^{-1}day^{-1})
2
1
0
−1
400
800
1200
1600
Mean annual precipitation (mm)
Potential
B
1
0
−1
0
10
20
Temperature of the wettest quarter (°C)
Realized
C
4
2
0
−2
Mean annual precipitation (mm)
Realized
D
Temperature of the wettest quarter (°C)
Realized
E
10
20
30
Soil clay content (%)
Realized
F
500
1000
Microbial biomass (g^{-1} soil)

Figure 4

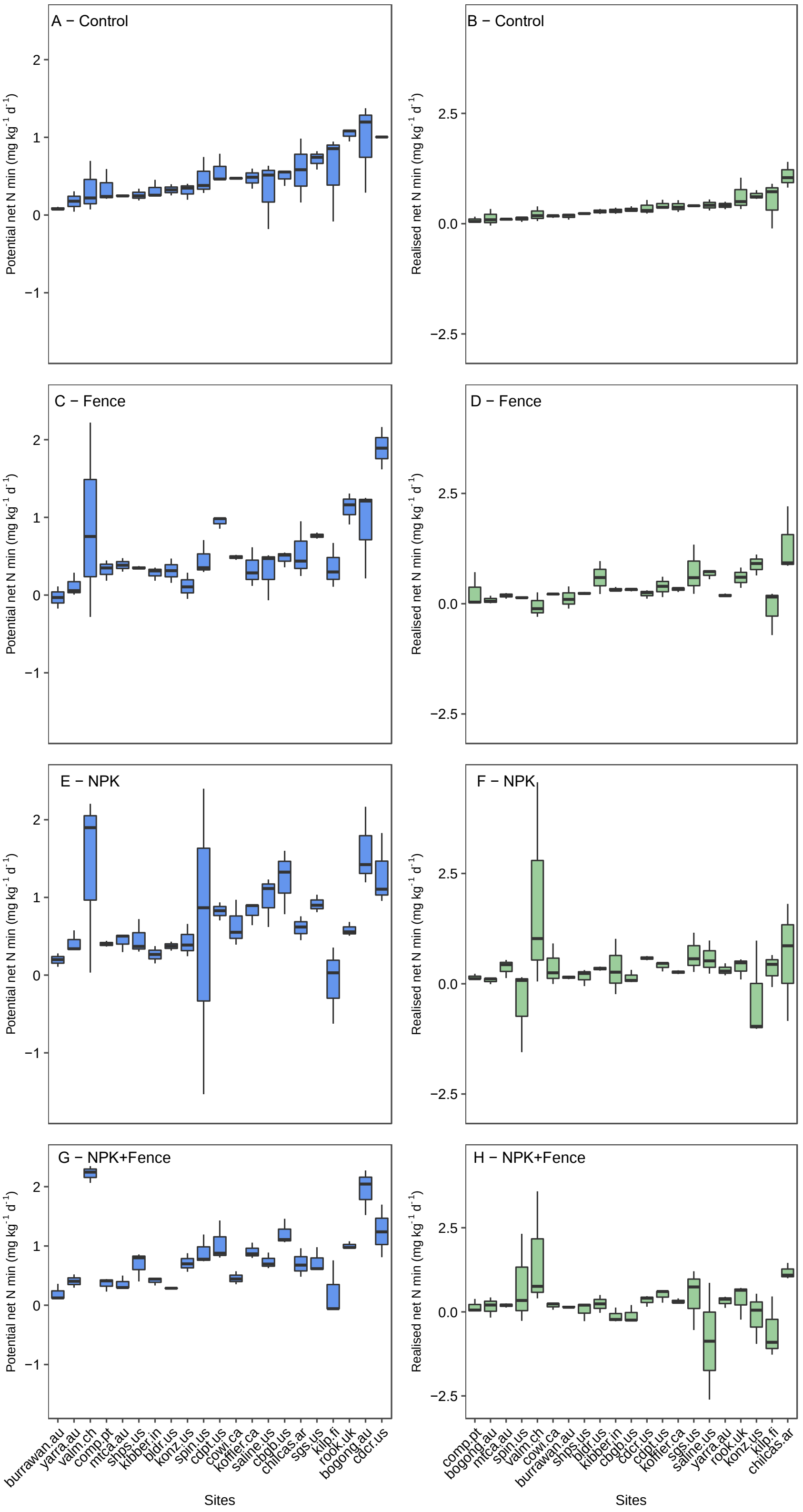
Supplementary Figures & Tables



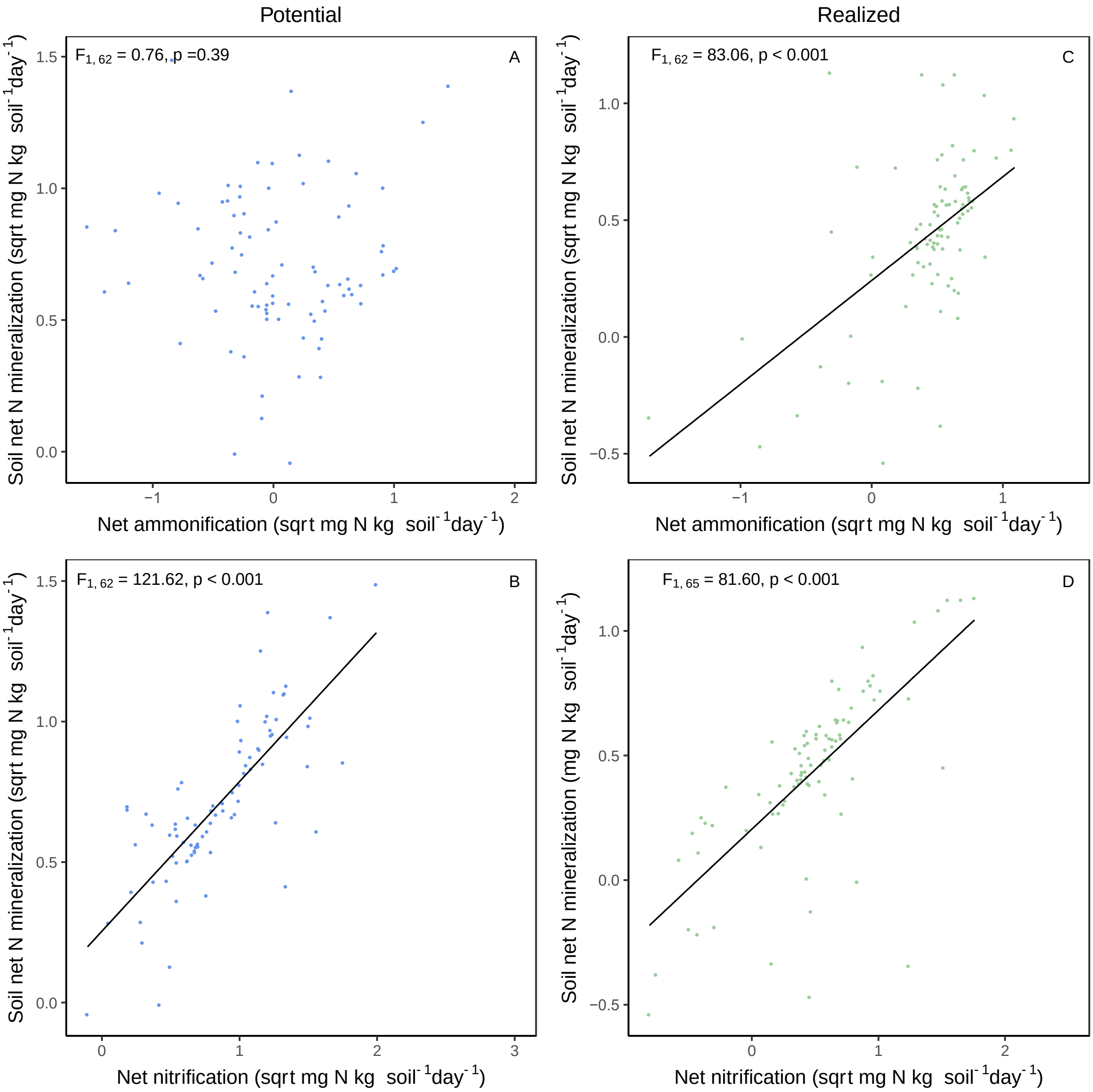
Supplementary Figure 1. Sampling design of the NutNet study. A) completely randomized block design, B) 5 m x 5 m sampling plots with 1 m x 1 m subplots. We only sampled from four treatments: C (green) = Control, F (yellow) = Fenced to remove herbivores, NPK (orange) = Fertilized with N, P, K and micronutrients, NPK+Fence (blue) = Fertilized with N, P, K and micronutrients and fenced to remove herbivores. Soil for this study were sampled from a 1 m x 1 m subplot randomly assigned for soil sampling (marked in red in B).



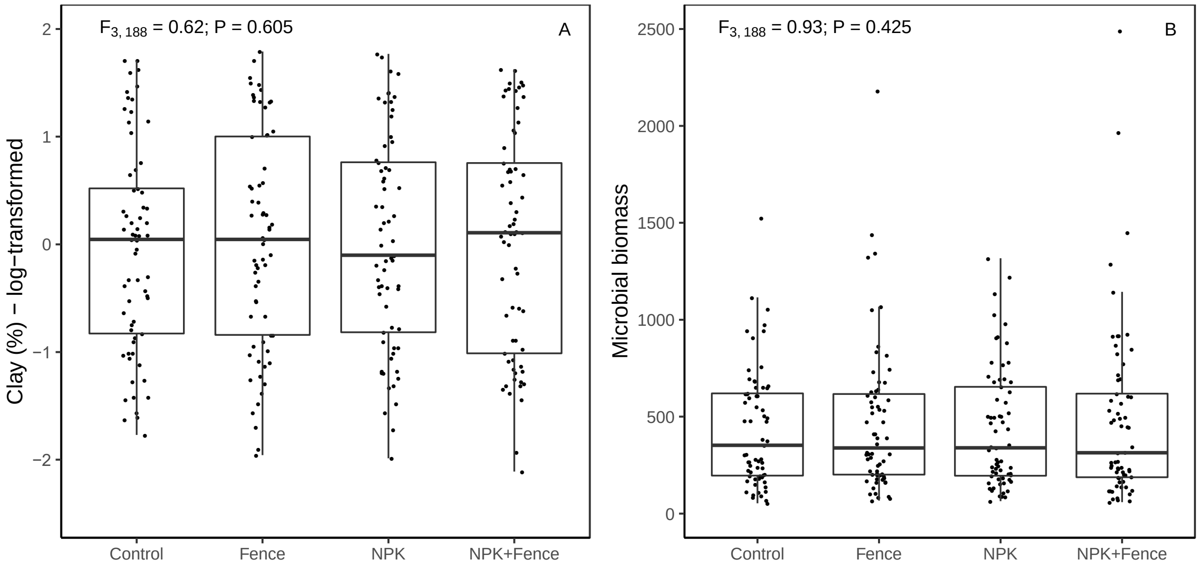
Supplementary Figure 2. Conceptual model of the expected causal relationships between treatments, environmental variables, soil properties and potential or realized soil net N_{min}. The conceptual model is based on hypotheses derived from the literature. MAP = mean annual precipitation, T.q.wet = temperature of the wettest quarter, Micr. Biom. = microbial biomass, Clay content = soil clay content, Micr. Biom. = soil microbial biomass, Control = control plots, Fence = herbivores removed, NPK = fertilized with N, P, K and micronutrients, NPK+Fence = fertilized with N, P, K and micronutrients and herbivores removed. Interaction effects are depicted with arrows pointing to solid blue dots.



Supplementary Figure 3. Global patterns in potential and realized soil net N_{min} across global grasslands. A, C, E, G) potential soil net N_{min} ordered according to the median value of each site in the control plots (A), n = 21 sites. B, D, F, H) realized soil net N_{min} ordered according to the median value of each site in the control plots (B), n = 22 sites. Boxplots show the median (50th percentile), 25th and 75th percentile of the data for each site. The whiskers represent 1.5 times the inter-quartile range. Control = control plots, Fence = herbivores removed, NPK = fertilized with N, P, K and micronutrients, NPK+Fence = fertilized with N, P, K and micronutrients and herbivores removed.



Supplementary Figure 4: Relationship between potential soil net N mineralization and A) net ammonification, and B) net nitrification and between realized soil net N mineralization and C) net ammonification and D) soil net nitrification. Points represent means per site and per treatment. The statistics are based on LMMs where potential and realized soil net N mineralization are dependent, soil ammonification/nitrification the independent variables. Site was included as random effect. All values were square-root transformed.



Supplementary Figure 5: Treatment effects on soil properties across 22 grasslands globally A) soil clay content, B) soil microbial biomass. Control = control plots, Fence = herbivores removed, NPK = fertilized with N, P, K and micronutrients, NPK+Fence = fertilized with N, P, K and micronutrients and herbivores removed. Soil clay content values are log transformed. Boxplots show the median (50th percentile), 25th and 75th percentile of the data across sites. Individual measures are shown in the background.

Supplementary Table 1. Site, continent of site location, country of site location, grassland type, elevation, latitude (in °), longitude (in °), mean annual precipitation (MAT, in °C), mean annual precipitation (MAP, in mm) and principal investigator(s) of the 22 Nutrient Network sites included in this study. Years = years of treatment application. Do. herb = indication of whether domestic herbivores were present (Yes) or not (No). Descriptions of the range of soil edaphic conditions between our sites can be found within the main text and Supplementary Figure S2. AR = Argentina, US = United States of America, AU = Australia, PT = Portugal, CA = Canada, CH = Switzerland, IN = India, FI = Finland, UK = United Kingdom, Years = years since treatment start. # = refers to numbers shown in Figure 1.

#	**Site**	**Continent**	**Country**	**Grassland type**	**Elevation**	**Latitude**	**Longitude**	**MAT**	**MAP**	**Years**	**Do. herb**	**Site PI**
1	bldr.us	North America	US	shortgrass prairie	1633	39.97	-105.23	9.7	425	5	No	Davis/Melbourne
2	bogong.au	Australia	AU	alpine grassland	1760	-36.87	147.25	5.7	1592	6	No	Moore/Morgan
3	burrawan.au	Australia	AU	semiarid grassland	425	-27.73	151.14	18.4	683	7	Yes	Firn/Buckley
4	cbgb.us	North America	US	tallgrass prairie	275	41.79	-93.39	9	855	6	No	Biederman/Harpole
5	cdcr.us	North America	US	tallgrass prairie	270	45.43	-93.21	6.3	750	8	No	Borer/Seabloom
6	cdpt.us	North America	US	shortgrass prairie	965	41.20	-101.63	9.5	445	9	No	Knops
7	chilcas.ar	South America	AR	mesic grassland	15	-36.28	-58.27	15.1	925	2	No	Yahdijan/Tognetti
8	comp.pt	Europe	PT	annual grassland	200	38.00	-8.00	16.5	554	3	No	Caldeira
9	cowi.ca	North America	CA	old field	50	48.46	-123.38	9.8	764	7	No	MacDougall
10	kibber.in	Asia	IN	alpine grassland	4241	32.32	78.01	1.1	504	3	Yes	Sankaran
11	kilp.fi	Europe	FI	tundra grassland	700	69.05	20.83	-4.1	551	2	No	Eskelinen/Virtanen
12	koffler.ca	North America	CA	pasture	301	44.02	-79.54	6.4	815	4	No	Cadotte
13	konz.us	North America	US	tallgrass prairie	440	39.07	-96.58	11.9	877	7	No	Blair/Smith/Komatsu
14	mtca.au	Australia	AU	savanna	285	-31.78	117.61	17.3	330	7	Yes	Prober
15	rook.uk	Europe	GB	mesic grassland	60	51.41	-0.64	9.8	706	6	No	Crawley
16	saline.us	North America	US	mixed grass prairie	440	39.05	-99.10	11.8	607	7	No	Smith/Komatsu
17	sgs.us	North America	US	shortgrass prairie	1650	40.82	-104.77	8.4	365	6	No	Blumenthal/Brown/Klein
18	shps.us	North America	US	shrub steppe	910	44.24	-112.20	5.5	262	9	Yes	Adler
19	spin.us	North America	US	pasture	271.3	38.14	-84.50	12.5	1140	7	No	McCulley
20	ufrec.us	North America	US	grassland	26	27.43	-81.91	22.3	1299	2	No	Silveira
21	valm.ch	Europe	CH	alpine grassland	2320	46.63	10.37	0.3	1098	7	No	Risch/Schütz
22	yarra.au	Australia	AU	mesic grassland	19	-33.61	150.73	17.2	898	2	No	Power

Supplementary Table 2: Soil edaphic properties at our 22 globally distributed sites on six continents. Site, continent of the site location, soil organic C content (Corg; %), soil total N content (Ntot; %), soil C:N ratio, soil pH, soil sand content (sand; %), soil silt content (Silt; %), soil clay content (Clay; %), water holding capacity (WHC; vol%), and soil bulk density (BD, g cm^{-3}). Description of mean annual precipitation and temperature, elevation, grassland type and the coordinates of each site can be found in Supplementary Table S1. # = refers to numbers shown in Figure 1.

#	Site	Continent	Corg	Ntot	C:N	pH	Sand	Silt	Clay	WHC	BD
1	bldr.us	North America	0.9	0.1	11.7	5.7	73.2	15.1	11.8	28.6	1.4
2	bogong.au	Australia	6.1	0.4	14.7	3.8	71.2	13.2	15.7	49.6	0.8
3	burrawan.au	Australia	0.9	0.1	16.4	4.7	82.5	12.0	5.5	26.3	1.4
4	cbgb.us	North America	0.8	0.1	11.1	5.5	88.4	7.3	4.4	25.0	1.1
5	cdcr.us	North America	1.6	0.1	14.9	5.0	90.4	6.7	2.9	26.0	1.0
6	cdpt.us	North America	1.1	0.1	11.2	5.6	76.4	13.7	9.9	37.6	1.3
7	chilcas.ar	South America	4.0	0.4	10.9	5.5	48.2	42.5	9.3	42.1	0.8
8	comp.pt	Europe	1.2	0.1	13.8	4.4	79.8	15.6	4.6	24.7	1.4
9	cowi.ca	North America	5.7	0.4	13.0	4.9	58.7	23.6	17.7	33.5	0.6
10	kibber.in	Asia	3.3	0.2	21.5	7.6	38.9	36.8	24.3	33.1	1.1
11	kilp.fi	Europe	7.8	0.6	13.5	3.9	59.8	28.5	11.7	57.0	0.6
12	koffler.ca	North America	2.6	0.2	11.1	6.9	62.8	27.9	9.4	30.7	1.0
13	konz.us	North America	3.9	0.3	14.3	5.6	15.6	49.4	35.0	43.2	0.9
14	mtca.au	Australia	0.8	0.1	15.4	4.4	82.9	10.5	6.6	22.5	1.4
15	rook.uk	Europe	3.2	0.3	12.3	3.4	83.3	10.7	6.0	41.0	1.1
16	saline.us	North America	4.1	0.3	15.1	6.7	26.8	44.3	28.9	35.2	1.1
17	sgs.us	North America	1.1	0.1	10.7	5.1	72.6	15.2	12.2	37.7	1.2
18	shps.us	North America	2.5	0.2	13.1	7.5	50.5	34.7	14.9	44.7	1.2
19	spin.us	North America	2.2	0.2	9.1	5.6	14.8	56.7	28.6	43.1	1.1
20	ufrec.us	North America	3.9	0.2	19.4	3.5	94.2	1.4	4.4	45.2	1.1
21	valm.ch	Europe	4.5	0.3	13.3	4.9	68.0	22.4	9.6	37.7	0.9
22	yarra.au	Australia	0.9	0.1	11.4	4.5	80.1	15.6	4.3	29.6	1.2

Supplementary Table 3. Description of exceptions to the fence design; sites not included in this list have standard design.

Site	Fence exception description	Herbivores excluded
cdpt.us	1.5 m of 10 cm cattle panels, with hardware cloth of up to 50 cm from ground level	Wild herbivores
shps.us	Similar to NutNet standard but top strand at 1.2 m	Sheep, wild herbivores
spin.us	Similar to NutNet standard but with two modifications: 3.6 cm hardware cloth and 1.5 m high fences	Wild herbivores
valm.ch	2.7 m wooden poles (25 cm diameter) driven 70 cm into ground, 3 m apart, covered with 5 cm square mesh to 2 m high and with extra cabling and supports to prevent snow damage. Fences enclose 6 m x 7 m area.	Wild herbivores

Supplementary Table 4. Overall model results for linear mixed effect models with Control, Fence, NPK, NPK+Fence as fixed effects. Block nested within site was included as a random effect. SE = standard error, df = degree of freedom.

	Estimate	**SE**	**DF**	**t-value**	**p-value**	**Estimate**	**SE**	**DF**	**t-value**	**p-value**
	Potential soil net N mineralization					*Realized soil net N mineralization*				
Intercept	0.635	0.060	179	10.663	<0.000	0.519	0.047	189	11.111	<0.000
Fence	-0.002	0.054	179	-0.043	0.966	-0.025	0.038	189	-0.638	0.524
NPK	0.116	0.054	179	2.160	0.032	-0.117	0.076	189	-1.531	0.127
NPK+Fence	0.197	0.054	179	3.686	<0.000	-0.210	0.084	189	-2.499	0.013
	Potential soil net ammonification					*Realized soil net ammonification*				
Intercept	0.132	0.125	179	1.062	0.290	0.619	0.040	189	15.555	<0.000
Fence	-0.029	0.089	179	-0.327	0.744	-0.107	0.061	189	-1.770	0.078
NPK	-0.133	0.088	179	-1.513	0.132	-0.253	0.103	189	-2.446	0.015
NPK+Fence	-0.208	0.088	179	-2.368	0.019	-0.389	0.113	189	-3.434	0.001
	Potential soil net nitrification					*Realized soil net nitrification*				
Intercept	0.725	0.082	179	8.885	<0.000	0.464	0.086	189	5.392	<0.000
Fence	0.010	0.058	179	0.175	0.861	0.032	0.044	189	0.731	0.466
NPK	0.233	0.057	179	4.067	<0.000	0.011	0.068	189	0.160	0.873
NPK+Fence	0.305	0.057	179	5.310	<0.000	-0.027	0.085	189	-0.315	0.753

Supplementary Table 5: Details of author contributions. Devel = developed, Contrib. = contributed.

Name	**Institution & Address**	**email**	**Devel. research question (s)**	**Ana-lyzed samples**	**Ana-lyzed data**	**Contrib. to data analyzes**	**Wrote paper**	**Contrib. to paper writing**	**Site coor-dinator**	**Nutrient Network coordinator**
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