Nitrogen and phosphorus addition-induced soil and microbial ecological stoichiometry regulated carbon decomposition and accumulation and reduced soil carbon content in Tibetan alpine meadows

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Abstract

Nitrogen (N) and phosphorus (P) additions to grasslands increase aboveground plant biomass and modify plant community composition, thereby affect plant-derived organic carbon inputting to soil and soil C cycling and storage. However, the effects of nutrient additions on SOC decomposition and soil C sequestration have no census and their underlying mechanisms are poorly understood. This study aimed to explore the mechanisms underlying SOC decomposition and SOC content decline in the topsoil of Tibetan alpine meadows after nine-year field N and P additions. Soil and microbial stoichiometric characteristics were measured and priming effects (PEs), substrate decomposition, as well as microbial C use efficiency (CUE) by adding 13C labeled substrate (glucose or vanillin) were analyzed. N and P additions differentially affected the magnitude and direction of PEs and SOC decomposition, accelerated substrate mineralization of glucose by 33-45% and that of vanillin by 11-45%, but decreased microbial CUE of glucose by 9-15% and that of vanillin by 11-48%. This was associated with the N and P additions-induced lower soil ecological stoichiometric ratios and higher microbial C:N:P ratios compared with Control. Therefore, these comprehensive effects of N and P additions on decomposition of SOC and plant-derived C substrates reduced SOC sequestration and thus SOC content. Long-term N and P additions would weaken soil functioning as C pool of Tibetan alpine meadows.

1. INTRODUCTION

Plant inputs as shoot or root litter is the predominant source of carbon to soil organic carbon (SOC). However, this input of plant-derived C substrate can strongly but differentially affect the process of SOC sequestration and loss due to their biochemical quality (Jackson et al., 2017; Sokol et al., 2019; Zhu et al., 2018). For example, the inputs of the exogenous organic C substrates to soil can increase or decrease the decomposition of soil organic matter (SOM) and such effects are known as priming effects (PEs) (Kuzyakov, 2010). However, the composition of plant-derived fresh organic matter (FOM) varies from simple and easily degraded compounds such as amino acids, sugars, and peptides, to recalcitrant complex compounds such as cellulose, hemicellulose, lignin, and proteins (Baumann et al., 2009; Sokol et al., 2019). In general, the easily degraded compounds like glucose, greatly enhance the decomposition of native SOC compared with the complex compounds like those plant litter containing relatively high lignin or high C:N (Blagodatskaya et al., 2007; Kuzyakov & Bol, 2006; Kuzyakov et al., 2000; Qiao et al., 2014). These findings indicated that the simpler structure of C substrate, the fast energy turnover; the more complex structure of C substrate, the lower PE is produced (Aye et al., 2018; Di Lonardo et al., 2017; Nottingham et al., 2009).

The PE could be generated by two different mechanisms (Fontaine et al., 2003; Kuzyakov et al., 2000): (1)

"Co-metabolism" or "stoichiometric decomposition" theory (Blagodatskaya et al., 2014; Kuzyakov et al., 2000; Razanamalala et al., 2018) and (2) "nutrient mining" theory (Craine et al., 2007; Razanamalala et al., 2018; Wang et al., 2015). "Co-metabolism" resulting from the release of extracellular enzymes by FOM decomposers helps to break down young SOM that has a structure similar to FOM, such as decaying vegetal tissues with a high C:N:P ratio. "Nutrient mining" targets an old SOM that is already transformed and rich in nutrients, with a long residence time. However, both mechanisms might coexist in the same soil (Chen et al., 2014; Fontaine et al., 2004; Razanamalala et al., 2018) and the balance between the two mechanisms was driven by the nutrient content of the soil solution (Chen et al., 2014) or SOM pool (Razanamalala et al., 2018).

Nutrient supply, especially N and P availability, strongly affect C cycling and storage in grasslands ecosystem (Li et al., 2018b; Luo et al., 2020; Ramirez et al., 2010; Riggs & Hobbie, 2016). N and P additions increase the above-ground plant biomass (Borer et al., 2017; Li et al., 2014; Isbell et al., 2013), increase or decrease biomass allocation to roots (Fornara & Tilman, 2012; Li et al., 2018b). These modifications in plant biomass would in turn affect C input to soil and SOC dynamics, however, the effects of nutrient additions on SOC decomposition and soil C sequestration had no census. For example, N additions increased SOC sequestration in prairie grasslands (Adair et al., 2009; Fornara & Tilman, 2012; Fornara et al., 2013) and sandy grasslands (Reid et al., 2012). Soudzilovskaia et al. (2007) reported NP addition increased litter production of graminoids with low decomposibility, thus increased SOC content in alpine tundra. Mack et al. (2004) showed that NP fertilization decreased SOC pools in arctic tundra. N and P additions accelerated SOM decomposition and reduced SOC content in the topsoil of Tibetan alpine meadows (Li et al., 2018a; Luo et al., 2019; Luo et al., 2020). The litter quality and various biomass allocations to aboveground plant and roots in different grassland ecosystems may resulted in varies in soil C sequestration and SOC storage due to nutrient additions (Soudzilovskaia et al., 2007; Fornara & Tilman, 2012; Li et al., 2018b). Besides these reasons, the inconsistent effects might be due to the differential response of microbial composition and their activity (Leff et al., 2015; Riggs & Hobbie, 2016; Zhang et al., 2018) and thereby SOM decomposition (Chen et al., 2014; Leff et al., 2015; Li et al., 2018a; Riggs et al., 2015) to N and P additions.

N and P additions increase or decrease microbial biomass (Li et al., 2018a; Liu & Greaver 2010; Ramirez et al., 2012), alter microbial C use efficiency (CUE) (Riggs & Hobbie, 2016; Luo et al., 2020) and microbial mineralization and soil C dynamics (Li et al., 2018a; Ramirez et al., 2012; Riggs et al., 2015). Microbes can adjust CUE to maintain their biomass stoichiometry according to stoichiometric ratio of substrates they feed on and soil nutrient content (Craine et al., 2007; Chen et al., 2014; Chen et al., 2016; Cleveland & Liptzin, 2007; Razanamalala et al., 2018) and thereby regulate the fate of plant-derived-C and C sequestration in soils (Geyer et al., 2016; Zhu et al., 2018). Microbial activity is driven by microbial demand for resources, with an average optimal C:N:P ratio of 60:7:1 for terrestrial ecosystems at the global level (Cleveland & Liptzin, 2007) and 48:5:1 for Tibetan alpine meadows (Chen et al., 2016; Zhao et al., 2017). Nutrient limitation can shift the microbial community composition from r-strategy microbes to K-strategy microbes which can decompose stable SOM to get access to available N or P and stimulate SOM mineralization, leading to a positive PE (Blagodatskaya & Kuzyakov, 2008; Chen et al., 2014; Zhu et al., 2018). Therefore, FOM decomposition and SOC mineralization due to the N and P additions can be explained using "stoichiometric decomposition" theory. Higher CUE intends an increased potential for C sequestration in soils while lower CUE implies relatively greater loss of C via microbial respiration (Riggs & Hobbie, 2016; Sokol et al., 2019).

The Qinghai-Tibetan Plateau is the highest and largest plateau on the earth. Alpine meadow is one of the dominant and most widely distributed grasslands type on the plateau, with much higher soil C content in the surface layer relative to other grasslands (Reid et al., 2012; Wen et al., 2013; Yang et al., 2008). Thus alpine meadows take an important role in maintaining soil functioning as C pool and sustaining ecological safety at regional or even global level (Yang et al., 2008; Wu et al., 2017). However, alpine meadows are particularly sensitive to climate change and anthropogenic activities (Fayiah et al., 2020; Liu et al., 2018). Anthropogenic drivers such as atmospheric deposition and nutrient addition increase the availability of nitrogen (N) and phosphorus (P) in grasslands, including alpine meadows (Galloway et al. 2008; Liu et al., 2013). Previous studies from alpine meadows showed that input of nitrogen (N) and phosphorus

(P) modified litter quality through increasing above-ground plant productivity and altering plants species' dominance in community, in particular the grass *Elymus nutans* (Li et al., 2014; Li et al., 2018b). These modifications can change the availability of organic C and the decomposition of SOM, thus reduce SOC content (Li et al., 2018a; Li et al., 2018b; Luo et al., 2019; Luo et al., 2020), with the lowest SOC content under P additions, as compared to N and NP additions (Li et al., 2018a). However, the mechanisms of this SOC decline induced by nutrient addition are unclear. Furthermore, little is known about how N and P additions affect soil stoichiometric characteristics and their effects on C decomposition, including plant-derived FOM decomposition and recalcitrant SOC decomposition which plays an important role in SOC sequestration and soil C pool (Li et al., 2017). FOM decomposition and priming of SOC decomposition caused by increased litter input due to nutrient addition is a potential mechanism that can explain the SOC declines and their differences between N and P addition. In this study, we examined soil and microbial stoichiometric characteristics after long-term field N and P addition to alpine meadows and C decomposition by adding ^{13}C labeled substrate (glucose or vanillin). We hypothesized that: (1) N and P additions modify soil N or P limitation status and thus differentially affect PEs and exogenous C substrates decomposition (Blagodatskaya & Kuzyakov, 2008; Chen et al., 2014; Zhu et al., 2018). (2) Glucose induces positive PEs mainly by "nutrient mining" while vanillin generates negative PEs mainly by "stoichiometric decomposition" due to the differences in substrates' decomposability (Aye et al., 2018; Di Lonardo et al., 2017; Nottingham et al., 2009; Wang et al., 2015) and in ecological stoichiometric properties of soil and microorganisms resulted from N and P additions (Blagodatskaya et al., 2014; Craine et al., 2007; Kuzyakov et al., 2000; Razanamalala et al., 2018). (3) N and P additions-induced higher SOC decomposition and lower microbial CUE lead to lower C accumulation and lower SOC content, with the highest C decomposition in P addition compared to N and NP additions due to P addition-induced severe N limitation, i.e., lower soil and microbial N:P (Blagodatskaya et al., 2014; Craine et al., 2007; Kuzyakov et al., 2000; Razanamalala et al., 2018).

2. MATERIALS AND METHODS

2.1 Study site description and experimental design

A long-term field nutrient addition experiment was carried out in Hezuo, Gansu, eastern Qinghai-Tibetan Plateau of China (N34°55', E102°53', 3,000 m above sea level). Hezuo has a mean annual temperature of 2.4°C and a 30-yr mean annual precipitation of 550 mm (1986 to 2015; Institute of Hezuo Meteorology). The site belongs to a typical alpine meadow with soil and vegetation characteristics as described previously (Li et al., 2014).

The experiment consists of four treatments (N alone, P alone, NP together, and a control without any nutrient addition (Control)) with five, 5 m × 5 m, replicates. Nitrogen and/or P in the form of urea and sodium dihydrogen phosphate anhydrous were applied annually (10 g m^{-2} yr.$^{-1}$) since 2009. For a complete description of the experimental design and establishment see Li et al. (2014).

Soil samples of 500 g were sampled from a 0-20 cm depth randomly in each treatment plot in August 2018, sieved thru a 2-mm to remove roots, homogenized and divided into two portions. One portion was stored at 4 °C for determination of soil available N (AN), microbial biomass C (MBC), microbial biomass N (MBN), microbial biomass P (MBP) and for the priming experiment. The other portion was air dried and subsequently used for pH, SOC, TN and total P (TP) analysis.

2.2 Soil properties analysis

Soil properties, including SOC, TN, AN, TP, pH, MBC, MBN, and MBP were measured using standard methods as described in Li et al. (2014, 2018a), and Zhu et al. (2018).

2.3 Priming experiment and CO_2 analysis

A 32-day laboratory incubation experiment was conducted at 20 °C in the dark to determine SOC mineralization and microbial C dynamics with or without glucose/vanillin amendment (n=5). For each microcosm, moist soil (equivalent to 70 g dry mass) sieved through 2-mm was placed in a 500-ml glass flask and pre-incubated at 20 °C in the dark for one week. Subsequently, either a water solution with ^{13}C labeled glucose or vanillin, representing easily degraded and recalcitrant C substrates, respectively, (Sigma-Aldrich, uniformly labeled, 99 atom %, 89.4 µg C g^{-1} dry soil) or distilled water were sprayed onto the soil to reach a moisture content equivalent to 60% of its water holding capacity (WHC). Soil moisture was maintained at this level by weighing and spraying with distilled water regularly.

CO_2 concentrations were determined at 1, 2, 3, 4, 5, 7, 10, 14, 19, 22, 25, 32 days after the treatment application started. To differentiate SOC- and glucose or vanillin-derived CO_2, the $\delta^{13}C$ value of respired CO_2 was measured with an Isotope Analyzer (Picarro TOC-CRDS, US) after dilution with a standard CO_2 gas of known concentration and isotopic composition. The $\delta^{13}C$ abundance of respired CO_2 was corrected for the added CO_2 gas using a mass balance approach. At the end of 32-day incubation, soils were extracted to determine the MBC and the amount of ^{13}C derived from the added substrate that had been incorporated into microbial biomass. The extraction was performed using the chloroform fumigation extraction method and the isotopic composition was determined using an Isotope Analyzer (Picarro TOC-CRDS, US), as described in our previous studies by Li et al. (2014) and Li et al. (2018a).

The priming effect (PE) induced by substrate (glucose or vanillin) amendment (ug C g^{-1} soil) was calculated as described by Qiao et al. (2014):

$$PE= CO_2^{Total} - CO_2^{substrate} - CO_2^{No\ substrate}$$

Where CO_2^{Total}, $CO_2^{substrate}$, $CO_2^{No\ substrate}$ were the total CO_2evolved from soil with glucose or vanillin amendment, CO_2 evolved from glucose or vanillin, and CO_2 evolved from soil without glucose or vanillin amendment, respectively. The cumulative PE, substrate decomposition, and SOC decomposition was the sum of each corresponding index measured during the incubation period.

Net C balance (NCB) representing C accumulation was calculated as:

$$NCB = C_{in} - C_{soc} - C_{substrate}$$

Where C_{in}, C_{soc}, and $C_{substrate}$ were substrate-C inputting to the soil, SOC-C loss, and substrate-C loss, respectively.

Microbial CUE was calculated as:

$$CUE= MB^{13}C / (MB^{13}C + ^{13}CO_2)$$

Where $MB^{13}C$ is the amount of substrate C incorporated into the microbial biomass and $^{13}CO_2$is the substrate-C respired as CO_2. The $\delta^{13}C$ of total microbial biomass ($\delta^{13}C_{MBC}$) was determined using the following equation as described by Blagodatskaya et al. (2014):

$$\delta^{13}C_{MBC} = (\delta^{13}C_f .C_f - \delta^{13}C_{nf} .C_{nf}) / (C_f - C_{nf})$$

Where $\delta^{13}C_f$ and $\delta^{13}C_{nf}$ were the $\delta^{13}C$ values of the 24-h chloroform fumigated and non-fumigated samples, respectively, and C_f and C_{nf} were the amounts of C in the fumigated and non-fumigated K_2SO_4 samples, respectively.

2.4 Statistical analysis

The influence of N and P addition on the soil properties, cumulative PEs, glucose or vanillin mineralization, SOC mineralization, NCB, and microbial CUE were analyzed with a two-way MANOVA. When there were significant interactions between the addition of N and P, one-way ANOVA's was used to test the effect of nutrient addition treatment on soil properties, using a Tukey HSD post hoc test ($p < 0.05$). Linear or quadratic regression models were fitted to describe the relationships between C mineralization, microbial CUE and stoichiometric characteristics of soil and microorganisms. Statistical tests were performed in SPSS 16.0. Graphs were plotted using Sigma Plot 12.5.

3. RESULTS

3.1 Soil abiotic and biotic properties and stoichiometric characteristics

N and P additions differently affected soil abiotic and biotic properties (Table S1, Table S2). Nutrient additions increased soil AN, with the pattern of N> NP >P>Control, but decreased soil pH, SOC (Table S1). Compared with Control, P and NP addition decreased soil C:N (Figure 1). N addition increased C:P and N:P while NP and P addition decreased soil C:P and N:P. Nutrient additions increased microbial C:N while decreased microbial C:P and N:P, with the lowest C:P and N:P under P addition.

3.2 Priming effect and C substrate mineralization

Glucose amendments had positive cumulative PEs under N and P additions while negative PE under Control, with the highest PE under P addition (Figure 2a). Vanillin amendments had negative cumulative PE for all nutrient treatments, with the highest PE under P addition. The PEs with glucose amendment were significantly correlated with soil and microbial C:N, C:P, N:P (Table 1). However, there were no significant relationships between PEs with vanillin amendment and stoichiometric characteristics of soil and microorganisms (Table 1).

Compared with control, nutrient additions increased C substrate mineralization, with much higher mineralization of vanillin than that of glucose (Figure 2b). Specifically, glucose mineralization was increased by 45%, 36%, and 33% under N, P and NP addition, respectively (Figure 2b). Vanillin mineralization was increased by 11%, 40%, and 48% under N, P and NP addition, respectively. Both C substrates (glucose and vanillin) mineralization was significantly correlated with soil and microbial C:N, C:P, N:P (except glucose mineralization and soil C:N) (Table 2).

3.3 SOC decomposition and NCB

N and P additions differently affected SOC mineralization and NCB (Figure 3). Specifically, with glucose amendment SOC mineralization increased by 33% under P addition while decreased by 9%, and 7% under N, and NP addition, respectively (Figure 3a). SOC mineralization was significantly correlated with soil C:P, N:P, and microbial C:N, C:P and N:P (Table 2). When vanillin was amended, SOC mineralization was increased by 7% and 8% under P and NP addition but decreased by 11% under N addition (Figure 3a). SOC mineralization was significantly correlated with soil and microbial C:N, C:P, N:P (Table 2).

The NCB were positive for all nutrient treatments regardless of glucose or vanillin was amended (Figure 3b). Compared with control, the NCB with glucose amendment were increased by 7% and 6% under N and NP additions, respectively, while decreased by 40% under P addition. The NCB with vanillin amendment was increased by 17% under N addition but decreased by 36% and 42% under P and NP addition, respectively (Figure 3b).

3.4 Microbial CUE

Nutrient additions decreased microbial CUE of glucose-C and vanillin-C, with much higher CUE of glucose-C than that of vanillin-C in all the treatments (Figure 4). Compared with Control, N and P additions decreased CUE of glucose-C by 9-15%, and that of vanillin-C by 24-27%, with the lowest CUE of glucose-C under N addition and CUE of vanillin-C under P addition, respectively. Microbial CUE of both substrates C was significantly correlated with soil and microbial stoichiometric ratios (Table 3).

4. DISCUSSION

N and P additions differently affected the magnitude and direction of PEs, decomposition of plant-derived substrate and SOC, microbial CUE, and thus C accumulation. This was mainly associated with the N and P additions-induced differences in soil nutrient status and microbial ecological stoichiometric ratios, as well as differences in substrate decomposability due to changes in plant community composition and plant species' predominance. Glucose induced positive PEs while vanillin induced negative PEs. These results were in accordance with our hypotheses. However, the positive PEs with glucose amendment was mainly generated by "nutrient mining" while negative PEs with vanillin amendment was generated by the balanced two mechanisms "co-metabolism" and "nutrient mining". This partially supported our hypotheses. The comprehensive effects on C decomposition and accumulation led to declines in SOC contents in response to N and P additions.

4.1 Effect of N and P additions on SOC decomposition

The SOC decomposition was determined by microbial basic respiration and PEs due to exogenous substrates input to soil, of which the latter is a vital factor regulating SOC decomposition and content. Our data showed nutrient additions affected the magnitude and direction of PEs (Figure 2a). N and P addition promoted native SOC decomposition with the input of glucose, resulting in positive PE, while depressed native SOC decomposition with the input of vanillin, resulting in negative PE (Figure 2a). This was in accordance with our predictions and other studies that easily degradable substrate produce positive PE and complex C produce negative PE (Aye et al., 2018; Di Lonardo et al., 2017; Nottingham et al., 2009; Wang et al., 2015). The positive PE was mainly due to easily degradable C substrate was easier to be utilized by microbes than recalcitrant C substrate (Di Lonardo et al., 2017; Fontaine et al., 2011; Wang et al., 2015). The added substrates provided energy and promoted microbial growth, as shown in the higher MBC with glucose input than with vanillin input under corresponding N and P additions (Figure S1). In response to nutrient limitation under N and P addition, microbial community shifted their composition and structure and mineralized more native SOC to meet their biomass stoichiometric ratio requirements (Fontaine et al., 2011; Lin et al., 2019; Riggs & Hobbie, 2016; Zhu et al., 2018), which resulted in higher PEs of glucose than of vanillin and positive PEs with glucose amendment. The negative PE might be resulted from “preferential substrate utilization”, that microorganisms preferred utilize amended substrate vanillin rather than recalcitrant native SOC (Kuzyakov & Bol, 2006; Werth & Kuzyakov, 2010). The differential PEs among N and P addition might be resulted from nutrient additions-induced changes in N and P availability and soil stoichiometric characteristics which modified the N or P limitation status for microorganisms. The higher soil N:P under N addition but lower N:P under P and NP addition, as well as the lower microbial N:P compared with Control indicated that microbes were more of P limited under N addition but more of N limited under P and NP addition (Figure 1) (Chen et al., 2016; Craine et al., 2007; Zhao et al., 2017). This resulted in higher PE under N and P additions than Control, and the highest PE under P addition.

In our study, the differences in PEs among N and P additions were likely responsible for the differential SOC decomposition. In general, the higher PEs, the higher SOC decomposition was, as indicated by SOC decomposition pattern: P$>$ NP $>$N (Figure 3a). However, SOC decomposition with vanillin under N addition did not follow this pattern, with N $<$ NP (Figure 3a). This might be due to N addition-induced decrease in MBC and pH (Table S1) and modifications in microbial community structure (Lin et al., 2019; Leff et al., 2015; Riggs & Hobbie, 2016), which constrained microbial respiration (Li et al., 2018b; Riggs & Hobbie, 2016) and thus led to lower SOC decomposition.

The significant relationships between PE with glucose amendment and ecological stoichiometric ratios of soil and microorganisms suggested that PE was mainly generated by “nutrient mining”. However, the PE with vanillin amendment might be generated by the balanced two main mechanisms “co-metabolism” and “nutrient mining” (Fontaine et al., 2004; Chen et al., 2014; Razanamalala et al., 2018). Our results suggested the increased productivity and modified plant composition predominance due to N and P additions affected the magnitude and direction of PEs, and thus SOC decomposition in alpine meadows. The underlying mechanisms on PEs: “co-metabolism” theory and “nutrient mining” theory may occur simultaneously, depending on soil nutrient availability and stoichiometric properties, and the composition of C substrate (Craine et al., 2007; Wang et al., 2015; Razanamalala et al., 2018).

4.2 Effect of N and P additions on substrate decomposition

N and P addition increased the decomposition of added substrate with differential magnitude among N and P addition (Figure 2b). This might be might be due to two main reasons. Firstly, nutrient addition-induced changes in soil N availability (Table S1) and microbial stoichiometric ratios were associated with substrate mineralization (Table 2). Studies have shown that the higher N availability, the higher substrate (glucose) mineralization was (Craine et al., 2007; Fontaine et al., 2011; Paterson & Sim 2013). Thus, the increased N availability, with the highest value under N addition, led to the increased substrate decomposition in response to N and P addition and the highest glucose mineralization under N addition (Figure 2b). Secondly, the nutrient addition-induced changes in microbial composition and structure regulated substrate decomposition because of preferential substrate utilization (Blagodatskaya et al., 2007; Fontaine et al., 2011; Di Lonardo et al., 2017). Fungi have an advantage in breaking down complex polymers such as lignin and cellulose while bacteria prefer soluble organic C such as glucose (Wang et al., 2015; Fontaine et al., 2011). Therefore fungi are thought to be more important than bacteria in mediating long-term soil C sequestration (Fontaine et al., 2011; Blagodatskaya et al., 2014). The soil pH (above 6.7) in our study site (Table S1) and the higher microbial C:N under N addition than P and NP addition (Figure 1b) indicated bacterial dominance in microbial community and shifts in microbial composition and structure due to nutrient additions. Our recent results from the same study site also showed N and P additions increased bacterial and fungal abundance of those who decompose complex C and those bacteria, such as Actinobacteria, who utilize available C to grow fast (Li et al., 2020 submitted). Many other studies also reported nutrient addition-induced modification in microbial composition and structure (Blagodatskaya et al., 2007; Leff et al., 2015; Lin et al., 2019; Zeng et al., 2016) and N addition increased the relative abundance of fast growing bacteria (Leff et al., 2015; Zeng et al., 2016). Nutrient addition-induced increase in the abundance of bacteria and fungi would accelerate the decomposition of both easily degradable C and recalcitrant organic C, as shown by other studies (Diamond et al., 2019; Riggs & Hobbie, 2016). The increased substrate mineralization (Figure 2b) and decreased CUE (Figure 4) showed that long-term N and P additions resulted in greater losses of easily degraded C and recalcitrant organic C by microbial respiration and led to lower substrate C accumulation. The higher vanillin mineralization than glucose mineralization (Figure 2b) and higher CEU of glucose-C than vanillin-C (Figure 4) implied that N and P addition-induced changes in plant dominance and litter chemical structure would accelerate decomposition of both plant-derived substrates and soil-derived C and reduce SOC sequestration in alpine meadows.

4.3 Effects of N and P additions on C sequestration and their implication for alpine meadows

The positive NCB under N and P additions including the Control (Figure 3b) in our study indicated that C input to soil in the form of plant residue was higher than C output from SOC decomposition. This would increase SOC accumulation and soil C content in the Tibetan meadows, as shown by Yang et al. (2008). Our data did show an increasing SOC content compared with the initial SOC concentration of 3.38% (Li et al., 2018a) at the beginning of the field experiment nine-years ago. The lower NCB with vanillin amendment than that with glucose amendment in corresponding treatments (Figure 3b) suggested that recalcitrant C input due to changes in plant composition and predominance may decrease C accumulation and thereby SOC content. However, there was no consistent effect of N and P additions on NCB pattern between glucose amendment and vanillin amendment. The NCB pattern under N and P additions was in verse with the pattern of SOC decomposition. The NCB with glucose amendment under P addition and that with vanillin amendment under P and NP addition was much lower than Control (Figure 3b). Though the NCB with glucose was higher than Control, its effect on SOC accumulation might be offset by the larger extent increase in glucose mineralization and the PE over the time because glucose is more accessible to microorganisms than recalcitrant SOC (Blagodatskaya et al., 2014; Fontaine et al., 2004). The much lower NCB with glucose or vanillin amendment under P and NP additions than the Control suggested that P and NP addition would reduce C accumulation and thus SOC content. The higher NCB with glucose or vanillin amendment under N addition than the Control suggested a potential increasing SOC accumulation and SOC content. This was contrasted with our hypothesis and the decline in SOC content and storage. The seemingly contrary result suggested that the positive NCB and the potential increase in SOC content under N addition might merely be temporary, due to the shorter period of incubation in our experiment. It needed to be pointed out that the NCB here was based on a 32-day incubation period. The added substrate, especially glucose, remaining in soil would enter into the active C pool (Hou, 2018) that was most easily utilized by microorganisms (Blagodatskaya et al., 2014) and continue to stimulate the native SOM decomposition (Fontaine et al., 2004). Over time, the added glucose might be consumed but SOM decomposition would proceed and result in a net C loss (Fontaine et al., 2004).

Our previous studies from the same experimental site showed N and P additions increased plant above-ground plant biomass and modified plant community composition of alpine meadows from grasses and forbs dominated community to *E . nutans* dominated community (Li et al., 2014; Li et al., 2018b). These changes increased the C inputs to soil as plant residues, however, C accumulation, especially plant derived-C would be reduced and thereby decrease SOC content because of the increased C substrate decomposition and the decreased microbial CUE in response to N and P additions. The changes in plant litter quality through altering plant species' predominance and plant composition also affected SOC decomposition due to the PEs caused by differential chemical structure of substrates. Our data showed N and P additions had positive PEs with glucose amendment but negative PEs with vanillin amendment, the total SOC decomposition with glucose amendment under P addition was increased by 33% and that with vanillin amendment under P and NP addition was increased by 7% and 8%, respectively (Figure 3a). This also contributed to the lower SOC content under P addition (-9%) than N (-2%) and NP (-4%) addition compared with Control (Table S1) (Li et al., 2018a). Therefore, the comprehensive effects of N and P additions on decomposition of SOC and plant-derived C substrates reduced SOC sequestration and thus SOC content in alpine meadows (Figure 5).

Studies on Tibetan alpine meadows, including one of our recent studies from the same study site, also showed that N and P enrichment decreased SOC content in the surface layer by reducing recalcitrant organic C content and slow C pool and increasing labile SOC content (Hou, 2018; Li et al., 2020 submitted; Luo et al., 2019; Luo et al., 2020). Some other studies out of Tibetan Plateau also reported that nutrient additions lowered SOC pool in the Arctic tundra (Mack et al., 2004), stimulated microbial decomposition of SOM in arctic permafrost soil (Wild et al., 2014) and caused considerable soil C losses in many high-latitude Arctic and sub-Arctic regions (Crowther et al., 2019). Given that alpine meadows and high-latitude Arctic tundra had higher soil C content than other grasslands (Yang et al., 2008; Reid et al., 2012; Wen et al., 2013), anthropogenic drivers such as atmospheric deposition and nutrient additions would decrease SOC content and soil C storage. This would weaken soil functioning as C pool of alpine meadows or Arctic grasslands.

CONCLUSION

Nine years of N and P additions to alpine meadows differentially affected the magnitude and direction of PEs and SOC decomposition (except with vanillin amendment under N addition), accelerated substrates (glucose and vanillin) mineralization and but decreased the microbial CUE. This was mainly associated with the differences in soil and microbial C:N:P stoichiometric ratios, and substrates decomposability due to N and P additions. These changes indicated that long-term N and P additions resulted in greater losses of both easily degraded C and recalcitrant organic C by microbial respiration and led to lower C accumulation, thereby lower SOC content in the topsoil compared with Control, in particular P addition (Figure 5). Therefore N and P additions would weaken the soil functioning as a C pool of alpine meadows.

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REFERENCES

Adair, E. C., Reich, P. B., Hobbie, S. E., Knops, J. M. H. (2009). Interactive effects of time, CO_2, N, and diversity on total belowground carbon allocation and ecosystem carbon storage in a grassland community. *Ecosystems* , *12* , 1037-1052. https://doi.org/10.1007/s10021-009-9278-9

Aye, N. S., Butterly, C. R., Sale, P. W. G., & Tang, C. X. (2018). Interactive effects of initial pH and nitrogen status on soil organic carbon priming by glucose and lignocelluloses. *Soil Biology and Biochemistry* , *123* , 33-44. https://doi.org/10.1016/j.soilbio.2018.04.027

Baumann, K., Marschner, P., Smernik, R. J., & Baldock, J. A. (2009). Residue chemistry and microbial community structure during decomposition of eucalypt, wheat and vetch residues. *Soil Biology and Biochemistry* , *41* , 1966-1975. https://doi.org/10.1016/j.soilbio.2009.06.022

Blagodatskaya, E. V., Blagodatsky, S. A., Anderson, T. H., Kuzyakov, Y. (2007). Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. *Applied Soil Ecology* ,*37* , 95-105. https://doi.org/10.1016/j.apsoil.2007.05.002

Blagodatskaya, E., & Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils* , *45* , 115-131. http:// doi.org/ 10.1007/s00374-008-0334-y

Blagodatskaya, E., Khomyakov, N., Myachina, O., Bogomolova, I., Blagodatsky, S., Kuzyakov, Y. (2014). Microbial interactions affect sources of priming induced by cellulose. *Soil Biology and Biochemistry* , *74* , 39-49. http:// doi.org/10.1016/j. soilbio.2014.02.017

Borer, E. T., Grace, J. B., Harpole, W. S., MacDougall, A. S., Seabloom, E. W. (2017). A decade of insights into grassland ecosystem responses to global environmental change. *Nature Ecology and Evolution* ,*1* , 0118. https://doi.org/10.1038/s41559-017-0118

Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E., Kuzyakov, Y. (2014). Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. *Global Change Biology* ,*20* , 2356-2367. http:// doi.org/10.1111/gcb.12475

Chen, Y. L., Chen, L. Y., Peng, Y. F., Ding, J. Z., Li, F., Yang, G. B., Liu, L., Fang, K., Zhang, B. B., Wang, J., Yang, Y. H. (2016). Linking microbial C:N:P stoichiometry to microbial community and abiotic factors along a 3500-km grassland transect on the Tibetan Plateau. *Global Ecology and Biogeography* , *25* , 1416-1427. https://doi.org/10.1111/geb.12500

Cleveland, C. C., & Liptzin, D. (2007). C:N:P stoichiometry in soil: Is there a “Redfield ratio” for the microbial biomass?*Biogeochemistry* , *85* , 235-252. https://doi.org/10.2307/20456544

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., McDougall, A., Peri, P.L., Prober, S.M., Stevens, C.J., & Routh, D. (2019). Sensitivity of global soil carbon stocks to combined nutrient enrichment. *Ecology Letters* , *22* , 936-945. https://doi.org/10.1111/ele.13258

Di Lonardo, D. P., Boer, W. D., Klein Gunnewiek, P. J. A., Hannula, S. E., Van der Wal, A. (2017). Priming of soil organic matter: Chemical structure of added compounds is more important than the energy content.*Soil Biology and Biochemistry* , *108* , 41-54. https://doi.org/10.1016/j.soilbio.2017.01.017

Diamond, S., Andeer, P. F., Li, Z., Crits-Christoph, A., Burstein, D., Anantharaman, K., Lane, K. R., Thomas, B. C., Pan, C., Northern, T. R., & Banfield, J. F. (2019). Mediterranean grassland soil C-N compound turnover is dependent on rainfall and depth, and is mediated by genomically divergent microorganisms. *Nature microbiology* ,*4* , 1356-1367. https://doi.org/10.1038/s41564-019-0449-y

Fayiah, M., Dong S. K., Khomera, S. W., Ur Rehman, S. A., Yang, M. Y., & Xiao, J. N. (2020). Status and challenges of Qinghai-Tibet Plateau’s grasslands: an analysis of causes, mitigation measures, and way forward.*Sustainability* , *12* , 1099. http://doi.org/10.3390/su12031099

Fontaine, S., Bardoux, G., Abbadie, L., & Mariotti, A. (2004). Carbon input to soil may decrease soil carbon content. *Ecology Letters* ,*7* , 314-320. https://doi.org/10.1111/j.1461-0248.2004.00579.x

Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J. M. G., Maire, V., Mary, B., Revaillot, S., & Maron, P. A. (2011). Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. *Soil Biology and Biochemistry* , *43* , 86-96. https://doi.org/10.1016/j.soilbio.2010.09.017

Fontaine, S., Mariottib, A., Abbadie, L. (2003). The priming effect of organic matter: a question of microbial competition? *Soil Biology and Biochemistry* , *35* , 837-843. http://doi.org/10.1016/S0038-0717(03)00123-8

Fornara, D. A., Banin, L., & Crwley, M. J. (2013). Multi-nutrient vs. nitrogen-only effects on carbon sequestration in grassland soils.*Global Change Biology* , *19* , 3848-3857. https://doi.org/10.1111/gcb.12323

Fornara, D.A., & Tilman, D. (2012). Soil carbon sequestration in prairie grasslands increased by chronic nitrogen addition.*Ecology* , *93* , 2030-2036. https://doi.org/10.1890/12-0292.1

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z. C., Freney, J. R., Martinelli, L. A., Seitzinger, S. P., & Sutton, M. A. (2008). Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* , *320* , 889-892. http://doi.org/10.1126/science.1136674

Geyer, K. M., Kyker-Snowman, E., Grandy, A. S., Frey, S. D. (2016). Microbial carbon use efficiency: accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. *Biogeochemistry* , *127* , 173-188. http://doi.org/10.1007/s10533-016-0191-y

Hou, Y. L. (2018). Effect of fertilization on carbon components in soil aggregates and decomposition dynamic of soil organic carbon in alpine meadows on the Qinghai-Tibetan Plateau. Lanzhou: MSc Dissertation, Lanzhou University.

Isbell, F., Reich, P. B., Tilman, D., Hobbie, S. E., Polasky, S., & Binder, S. (2013). Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. *Proceedings of National Academy of Sciences* , *110* , 11911-11916. http://doi.org/10.1073/pnas.1310880110. http://doi.org/10.1073/pnas.1310880110

Jackson, R. B., Lajtha, K., Crow, S. E., Hugelius, G., Kramer, M. G., Piñeiro, G. (2017). The ecology of soil carbon: pools, vulnerabilities, and biotic and abiotic controls. *Annual Review of Ecology, Evolution, and Systematics* , *48* , 419-445. https://doi.org/10.1146/annurev-ecolsys-112414- 054234

Kuzyakov, Y. (2010). Priming effects: interactions between living and dead organic matter. *Soil Biology and Biochemistry* , *42* , 1363-1371. http://doi.org/10.1016/j.soilbio.2010.04.003

Kuzyakov, Y., & Bol, R. (2006). Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar.*Soil Biology and Biochemistry* , *38* , 747-758. https://doi.org/10.1016/j.soilbio.2005.06.025

Kuzyakov, Y., Friedel, J.K., & Stahr, K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry* , *32* , 1485-1498. https://doi.org/10.1016/S0038-0717(00)00084-5

Leff, J. W., Jones, S. E., Prober, S. M., Barberan, A., Borer. E. T., Firn, J. L., Harpole, W. S., Hobbie, S. E., Hofmockel, K. S., Knops, J. M. H., McCulley, R. L., Pierre, K. L., Risch, A. C., Seabloom, E. W., Schütz, M., Steenbock, C., Stevens, C. J., & Fierer, N. (2015). Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of National Academy of Sciences* , *112* , 10967-10972. http://doi.org/10.1073/pnas.1508382112.

Li, H., Yang, S., Xu, Z. W., Yan, Q. Y., Li, X. B., van Nostrand, J. D., He, Z. L., Yao, F., Han, X. G., Zhou, J. Z., Deng, Y., & Jiang, Y. (2017). Responses of soil microbial functional genes to global changes are indirectly influenced by aboveground plant biomass variation.*Soil Biology and Biochemistry* , *104* , 18-29. http://doi.org/ 10.1016/j.soilbio.2016.10.009

Li, J. H., Hou, Y. L., Zhang, S. X., Li, W. J., Xu, D. H., Knops, J. M. H., & Shi, X. M. (2018a). Fertilization with nitrogen and/or phosphorus lowers soil organic carbon sequestration in alpine meadows. *Land Degradation & Development* , *29* , 1634-1641. http://doi.org/10.1002/ldr.2961

Li, J. H., Li, F., Chen, S., Li, W. J., Abbott, L. K., & Knops, J. M. H. (2018b). Nitrogen additions promote decomposition of soil organic carbon in a Tibetan alpine meadow. *Soil Science Society of America Journal* , *82* , 614-621. http://doi.org/10.2136/sssaj2017.12.0417

Li, J. H., Yang, Y. J., Li, B. W., Li, W. J., Wang, G., & Knops, J. M. H. (2014). Effects of nitrogen and phosphorus fertilization on soil carbon fractions in alpine meadows on the Qinghai-Tibetan plateau. PLoS One, 9(7), e103266. https://doi.org/10.1371/journal.pone.0103266

Lin, Y. X., Ye, G. P., Kuzyakov, Y., Liu, D. Y., Fan, J. B., & Ding, W. X. (2019). Long-term manure application increases soil organic matter and aggregation, and alters microbial community structure and keystone taxa. *Soil Biology and Biochemistry* , *134* , 187-196. http://doi.org/10.1016/j.soilbio.2019.03.030

Liu, X. J., Zhang, Y., Han, W. H., Tang, A. H., Shen, J. L., Cui, Z. L., Vitousek, P., Erisman, J. W., Goulding, K., Christie, P., Fangmeier, A., & Zhang, F. S. (2013). Enhanced nitrogen deposition over China. Nature 494(7438):459-462. https://doi.org/10.1038/nature11917

Liu, L. L., & Greaver, T. L. (2010). A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecology Letters* , *13* , 819-828. http://doi.org/10.1111/j.1461-0248.2010.01482.x

Liu, S., Zamanian, K., Schleuss, P., Zarebanadkouki, M., & Kuzyakov, Y. (2018). Degradation of Tibetan grasslands: consequences for carbon and nutrient cycles. *Agriculture Ecosystem & Environment* ,*252* , 93-104. http://doi.org/10.1016/j.agee.2017.10.011

Luo, R. Y., Fan, J. L., Wang, W. J., Luo, J. F., Kuzyakov, Y., He, J. S., Chu, H. Y., & Ding, W. X. (2019). Nitrogen and phosphorus enrichment accelerates soil organic carbon loss in alpine grassland on the Qinghai-Tibetan Plateau. *The Science of the Total Environment* , *650* , 303-312. https://doi.org/10.1016/j.scitotenv.2018.09.038

Luo, R. Y., Kuzyakov, Y., Liu, D. Y., Fan, J. L., Luo, J. F., Lindsey, S., He, J. S., & Ding, W. X. (2020). Nutrient addition reduces carbon sequestration in a Tibetan grassland soil: Disentangling microbial and physical controls. *Soil Biology and Biochemistry* , *144* , 107764. https://doi.org/10.1016/j.soilbio.2020.107764

Mack, M. C., Schuur, E. A. G., Bret-Harte, M. S., shaver G. S., & Chapin III, F. S. (2004). Ecosystem carbon storage in arctic tundra reduced by long term nutrient fertilization. *Nature* , *431* , 440-443. https://doi.org/10.1038/nature02887

Nottingham, A. T., Griffiths, H., Chamberlain, P. M., Stott, A. W., Tanner, E. V. J. (2009). Soil priming by sugar and leaf-litter substrates: A link to microbial groups. *Applied Soil Ecology* ,*42* , 83-90. https://doi.org/10.1016/j.apsoil.2009.03.003

Paterson, E., & Sim, A. (2013). Soil-specific response functions of organic matter mineralization to the availability of labile carbon.*Global Change Biology* , *19* , 1562-1571. https://doi.org/10.1111/gcb.12140

Qiao, N., Schaefer, D., Blagodatskaya, E., Zou, X., Xu, X., Kuzyakov, Y. (2014). Labile carbon retention compensates for CO_2released by priming in forest soils. *Global Change Biology* ,*20,* 1943-1954. http://doi.org/10.1111/gcb.12458.

Ramirez, K. S., Craine, J. M., & Fierer, N. (2010). Nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied. *Soil Biology and Biochemistry* , *42* , 2336-2338. http://doi.org/10.1016/j.soilbio.2010.08.032

Ramirez, K. S., Craine, J. M., & Fierer, N. (2012). Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology, 18, 1918-1927. https://doi.org/10.1111/j.1365-2486.2012.02639.x

Razanamalala, K., Fanomezana, R. A., Razafimbelo, T., Chevallier, T., Trap, J., Blanchart, E., & Bernard, L. (2018). The priming effect generated by stoichiometric decomposition and nutrient mining in cultivated tropical soils: Actors and drivers. *Applied Soil Ecology* , *126* , 21-33. https://doi.org/10.1016/j.apsoil.2018.02.008

Reid, J. P., Adair, E. C., Hobbie, S. E., Reich, P. B. (2012). Biodiversity, nitrogen deposition, and CO_2 affect grassland soil carbon cycling but not storage. *Ecosystems* ,*15* , 580-590. https://doi.org/10.1007/s10021-012-9532-4

Riggs, C. E., & Hobbie, S. E. (2016). Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. *Soil Biology and Biochemistry* , *99* , 54-65. http://doi.org/10.1016/j.soilbio.2016.04.023

Riggs, C. E., Hobbie, S. E., Bach, E. M., Hofmockel, K. S., & Kazanski, C. E. (2015). Nitrogen addition changes grassland soil organic matter decomposition. *Biogeochemistry* , *125* , 203-219. https://doi.org/10.1007/s10533-015-0123-2

Sokol, N. W., Sanderman, J., & Bradford, M. A. (2019). Pathways of mineral-associated soil organic matter formation: integrating the role of plant carbon source, chemistry, and point of entry. *Global Change Biology* , *25* , 12-24. https://doi.org/10.1111/gcb.14482

Soudzilovskaia, N. A., Onipchenko, V. G., Cornelissen, J. H. C., & Aerts, R. (2007). Effects of fertilization and irrigation on 'foliar afterlife' in alpine tundra. *Journal of Vegetation Science* ,*18* , 755-766. http://doi.org/10.1111/j.1654-1103.2007.tb02591.x.

Wang, H., Boutton, T. W., Xu, W. H., Hu, G. Q., Jiang, P., & Bai, E. (2015). Quality of fresh organic matter affects priming of soil organic matter and substrate utilization patterns of microbes. *Scientific Reports* , *5* , 10102 . http://doi.org/10.1038/srep10102

Wen, L., Dong, S. K., Li, Y. Y., Wang, X. X., Li, X. Y., Shi, J. J., & Dong, Q. M. (2013). The impact of land degradation on the C pools in alpine grasslands of the Qinghai-Tibet Plateau. *Plant and Soil* ,*368* , 329-340. http://doi.org/10.1007/s11104-012-1500-4

Werth, M., & Kuzyakov, Y. (2010). ^{13}C fractionation at the root-microorganisms-soil interface: a review and outlook for partitioning studies. *Soil Biology and Biochemistry* , *42* , 1372-1384. https://doi.org/10.1016/j.soilbio.2010.04.009

Wild, B., Schnecker, J., Alves, R. J. E., Barsukov, P., Barta, J., Capek, P., Gentsch, N., Gittel, A., Guggenberger, G., Lashchinsky, N., Mikutta, R., Rusalimova, O., Santruckova, H., Shibistova, O., Urich, T., Watzka, M., Zrazhevskaya, G., & Richter, A. (2014). Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil. *Soil Biology and Biochemistry* , *75* , 143-151. http://doi.org/10.1016/j.soilbio.2014.04.014

Wu, L. W., Yang, Y. F., Wang, S. P., Yue, H. W., Lin, Q.Y., Hu, Y.G. , He, Z. L., Nostrand, J. D. V., Hale, L., Li, X. Z., Gilbert, J. A., & Zhou, J. Z. (2017). Alpine soil carbon is vulnerable to rapid microbial decomposition under climate cooling. *The ISME Journal* , *11* , 2102-2111. https://doi.org/10.1038/ismej.2017.75

Yang, Y. H., Fang, J. Y., Tang, Y. H., Ji, C. J., Zheng, C. Y., He, J. S., & Zhu, B. (2008). Storage, patterns and controls of soil organic carbon in the Tibetan grasslands. *Global Change Biology* ,*14* , 1592-1599. https://doi.org/10.1111/j.1365-2486.2008.01591.x

Zeng, J., Liu, X. J., Song, L., Lin, X. G., Zhang, H. Y., Shen, C. C, & Chu, H. Y. (2016). Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biology and Biochemistry* , *92* , 41-49. http://doi.org/10.1016/j.soilbio.2015.09.018

Zhang, T. A., Chen, Y. H., & Ruan H. H. (2018). Global negative effects of nitrogen deposition on soil microbes. *The ISME Journal* ,*12* , 1817-1825. http://doi.org/10.1038/s41396-018-0096-y

Zhao, H., Sun, J., Xu, X. L., & Qin, X. J. (2017). Stoichiometry of soil microbial biomass carbon and microbial biomass nitrogen in China's temperate and alpine grasslands. *European Journal of Soil Biology* , *83* , 1-8. https://doi.org/10.1016/j.ejsobi.2017.09.007

Zhu, Z. K., Ge, T. D., Luo, Y., Liu, S. L., Xu, X. L., Tong C. L, Shibistova, O., Guggenberger, G., & Wu, J. S. (2018). Microbial stoichiometric flexibility regulates rice straw mineralization and its priming effect in paddy soil. *Soil Biology and Biochemistry* ,*121* , 67-76. https://doi.org/10.1016/j.soilbio.2018.03.003

Table 1 Correlations between priming effects (PEs) and stoichiometric properties of soil and microorganisms

PEs	Stoichiometric properties	Stoichiometric properties	Lineal correlations
Glucose	Soil	**C:N**	**$Y=-754.687+158.353X-8.245X^2$ ($R^2=0.408$, $p=0.0$**
		C:P	**$Y=108.194-4.076X+0.037X^2$ ($R^2=0.878$, $p<0.001$)**
		N:P	**$Y=105.759-39.586X+3.576X^2$ ($R^2=0.863$, $p<0.001$**
	Microbes	**C:N**	**$Y=-271.507+108.618X-10.592X^2$ ($R^2=0.740$, $p<0.$**
		C:P	**$Y=10.893-0.400X$ ($R^2=0.808$, $p<0.001$)**
		N:P	**$Y=9.772-1.633X$ ($R^2=0.828$, $p<0.001$)**
Vanillin	Soil	C:N	$Y=-73.728+5.547X$ ($R^2=0.085$, $p=0.212$)
		C:P	$Y=-12.115-0.112X$ ($R^2=0.044$, $p=0.376$)
		N:P	$Y=-9.913-1.575X$ ($R^2=0.072$, $p=0.253$)
	Microbes	C:N	$Y=-5.067-2.548X$ ($R^2=0.048$, $p=0.354$)
		C:P	$Y=-16.823-0.049X$ ($R^2=0.008$, $p=0.713$)
		N:P	$Y=-17.710-0.031X$ ($R^2=0.001$, $p=0.955$)

Table 2 Correlations between mineralization of carbon substrate and soil organic carbon (SOC) and stoichiometric characteristics of soil and microoganisms

C mineralization	Stoichiometric properties	Stoichiometric properties	Correlations
Glucose			
Substrate	Soil	C:N	**$Y=-76.199+16.507X-0.851X^2$ ($R^2=0.447$, p**
		C:P	**$Y=12.625-0.369X+0.004X^2$ ($R^2=0.538$, p=**
		N:P	**$Y=12.038-3.463X+0.336X^2$ ($R^2=0.531$, p=**
	Microbes	**C:N**	**$Y=-7.679+3.900X-0.323X^2$ ($R^2=0.967$, p<**
		C:P	**$Y=3.032+0.127X-0.003X^2$ ($R^2=0.852$, p<**
		N:P	**$Y=3.447+0.302X-0.039X^2$ ($R^2=0.923$, p<**
SOC	Soil	**C:N**	$Y=-541.629+118.510X-5.948X^2$ ($R^2=0.032$, p=0.
		C:P	**$Y=130.152-3.027X+0.026X^2$ ($R^2=0.517$, p**
		N:P	**$Y=133.445-31.040X+2.625X^2$ ($R^2=0.584$,**
	Microbes	**C:N**	**$Y=207.562+107.708X-11.151X^2$ ($R^2=0.29$**
		C:P	**$Y=70.322-2.549X+0.051X^2$ ($R^2=0.670$, p<**
		N:P	**$Y=65.700-9.112X+0.743X^2$ ($R^2=0.598$, p<**
Vanillin			
Substrate	Soil	**C:N**	**$Y=68.519-5.232X$ ($R^2=0.635$, $p<0.001$)**
		C:P	**$Y=24.084-0.162X$ ($R^2=0.767$, $p<0.001$)**
		N:P	**$Y=24.256-1.679X$ ($R^2=0.685$, $p<0.001$)**
	Microbes	**C:N**	**$Y=133.39860.535X-6.044X^2$ ($R^2=0.902$, p<**
		C:P	**$Y=19.648-0.186X$ ($R^2=0.917$, $p<0.001$)**
		N:P	**$Y=20.620-1.731X+0.093X^2$ ($R^2=0.891$, p<**
SOC	Soil	**C:N**	**$Y=697.192-122.748X+5.828X^2$ ($R^2=0.308$,**
		C:P	**$Y=35.364+1.032X-0.013X^2$ ($R^2=0.961$, p<**
		N:P	**$Y=38.778+9.098X-1.175X^2$ ($R^2=0.977$, p<**
	Microbes	**C:N**	**$Y=-147.433+85.683X-8.977X^2$ ($R^2=0.894$,**
		C:P	**$Y=64.676-1.264X+0.024X^2$ ($R^2=0.804$, p<**
		N:P	**$Y=63.450-5.374X+0.442X^2$ ($R^2=0.834$, p<**

Table 3 Correlations between microbial carbon use efficiency (CUE) and stoichiometric properties of soil and microorganisms

CUE	Stoichiometric properties	Stoichiometric properties	Correlations
Glucose			
	Soil	**C:N**	**$Y=7.524-1.401X +0.072X^2$(R^2=0.346, p=0.027)**
		C:P	**$Y=0.011+0.029X-0.001X^2$ (R^2=0.458, p=0.005)**
		N:P	**$Y=0.062+0.268X-0.027X$ (R^2=0.483, p=0.004)**
	Microbes	**C:N**	**$Y=1.103-0.063X$ (R^2=0.958, p<0.001)**
		C:P	**$Y=0.776-0.014X+0.001X^2$ (R^2=859, p<0.001)**
		N:P	**$Y=0.737-0.038X+0.004X^2$ (R^2=919, p<0.001)**
Vanillin			
	Soil	**C:N**	**$Y=6.961-1.407X+-0.074X^2$ (R^2=0.582, p=0.001)**
		C:P	**$Y=-0.597+0.034X-0.001X^2$ (R^2=0.795, p<0.001)**
		N:P	**$Y=-0.558+0.325X-0.030X^2$ (R^2=0.744, p<0.001)**
	Microbes	**C:N**	**$Y=2.476-0.856X+0.082X^2$ (R^2=920, p<0.001)**
		C:P	**$Y=0.251-0.003X-0.001X^2$ (R^2=910, p<0.001)**
		N:P	**$Y=0.214+0.014X$ (R^2=0.944, p<0.001)**

Figure captions

FIGURE 1 Effects N and P addition on stoichiometric characteristics of soil (a) and microorganisms (b) (means ± 1SE, n=5). Abbrevation: Control the control without any nutrient addition, N N-alone addition, P P-alone addition, NP N and P together addition. The statistics are from a two-way ANOVA, *, **, *** and ns denoting$p$ <0.05, p <0.01,p <0.001, and p >0.05, respectively. Letters indicate significant difference among N and P additions at$p$ < 0.05. Numbers above N, P, and NP are effects of N and P compared to Control

FIGURE 2 Effects N and P addition on priming effect (PE) with glucose or vanillin substrate amendment (a) and carbon substrate mineralization (b). Values shown are means ± 1SE (n=5 per treatment). Abbrevation: Control the control without any nutrient addition, N N-alone addition, P P-alone addition, NP N and P together addition. The statistics are from a two-way ANOVA, *** and ns denoting$p$ <0.001, and p >0.05, respectively. Letters indicate significant difference among N and P additions at$p$ < 0.05. Numbers above N, P, and NP bars are effects of N and P compared to Control

FIGURE 3 Effects N and P addition on soil organic carbon (SOC) decomposition (a), and soil net carbon balance (NCB) (b). Values are mean ± 1SE (n=5 per treatment). Abbrevation: Control the control without any nutrient addition, N N-alone addition, P P-alone addition, NP N and P together addition. The statistics are from a two-way ANOVA, *** denoting$p$ <0.001. Letters indicate significant difference among N and P additions at p < 0.05. Numbers above N, P, and NP bars are effects of N and P compared to Control

FIGURE 4 Effects of N and P additions on the microbial carbon use efficiency (CUE) (Mean ± 1SE, n=5) of ^{13}C-glucose and ^{13}C-vanillin. Abbrevation: Control the control without any nutrient addition, N N-alone addition, P P-alone addition, NP N and P together addition. The statistics are from a two-way ANOVA, *, *** and ns denoting p <0.05,p <0.001, and p >0.05, respectively. Letters indicate significant differences among N and P additions at$p$ < 0.05. Numbers above N, P, and NP bars are effects of N and P compared to Control

FIGURE 5 A conceptual diagram illustrating the main effects of N and P additions on C decomposition and accumulation in Tibetan alpine meadows. Abbrevation: SOC soil organic carbon, CUE microbial carbon use efficiency. The red, green and yellow symbols and numbers denote the processes influenced by N-alone addition, P-alone addition, and NP together addition, respectively. The short arrows —and — represent the increase or decrease in soil C processes in response to N and P additions, respectively. The numbers in red, green and yellow denote increase or decrease in % under N, NP and P addition compared with Control.

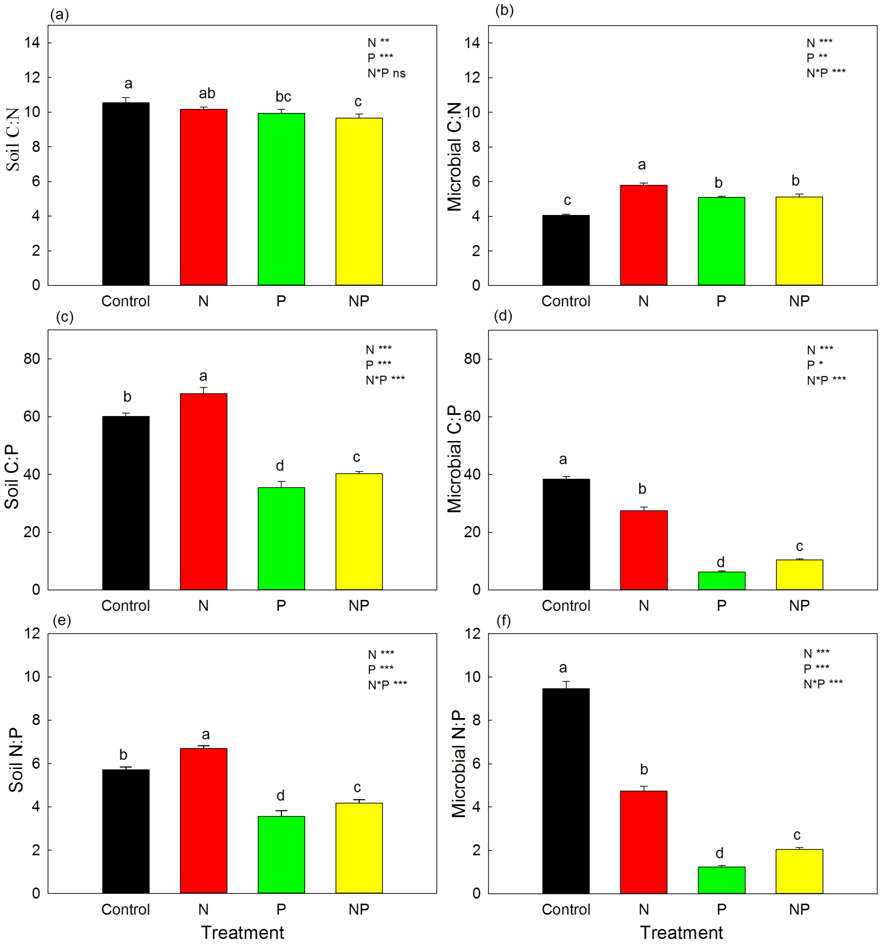
(a)
Soil C:N
N **
P ***
N*P ns
a
ab
bc
c
(b)
Microbial C:N
N ***
P **
N*P ***
c
a
b
b
(c)
Soil C:P
N ***
P ***
N*P ***
b
a
d
c
(d)
Microbial C:P
N ***
P *
N*P ***
a
b
d
c
(e)
Soil N:P
N ***
P ***
N*P ***
b
a
d
c
(f)
Microbial N:P
N ***
P ***
N*P ***
a
b
d
c
Control
N
P
NP
Treatment

Fig. 1

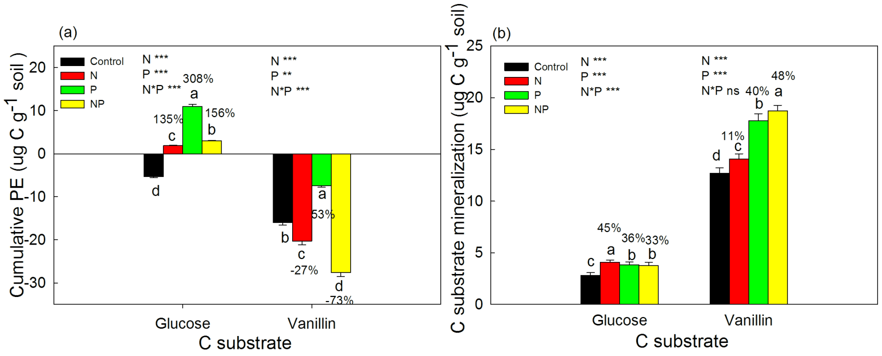
(a)
Cumulative PE (ug C g^{-1} soil)
Control
N
P
NP
N ***
P ***
N*P ***
N ***
P **
N*P ***
308%
135%
156%
a
c
b
d
b
c
-27%
a
53%
d
-73%
Glucose
Vanillin
C substrate
(b)
C substrate mineralization (ug C g^{-1} soil)
Control
N
P
NP
N ***
P ***
N*P ***
N ***
P ***
N*P ns
45%
36%
33%
c
a
b
b
11%
40%
48%
d
c
b
a
Glucose
Vanillin
C substrate

Fig 2

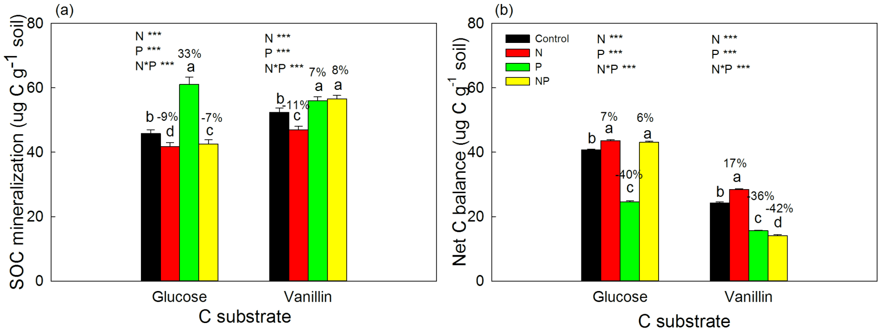

Fig 3

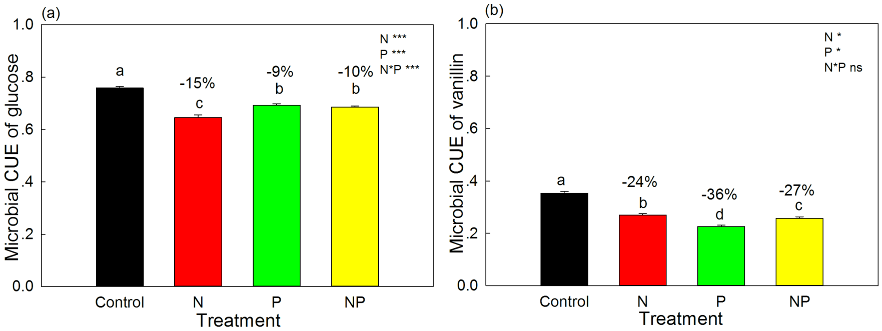

Fig 4

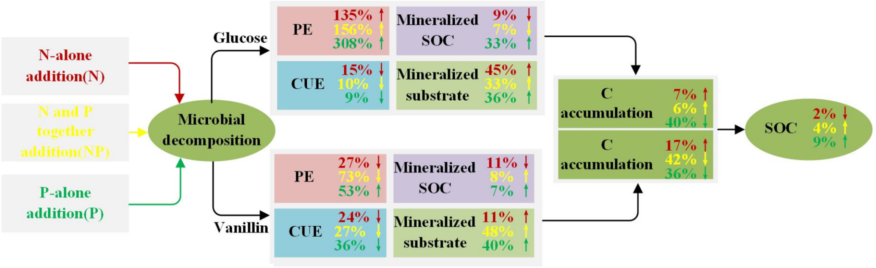

Fig 5