

FATE OF ORGANIC AND INORGANIC NITROGEN IN CRUSTED AND NON-CRUSTED *KOBRESIA* GRASSLANDSLi Zhang^{1,2,3}, Sebastian Unteregelsbacher², Silke Hafner², Xingliang Xu^{1*}, Per-Marten Schleuss², Georg Miede⁴, Yakov Kuzyakov^{1,2,5}¹Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources, Chinese Academy of Sciences, Beijing, PR China²Department of Soil Science of Temperate Ecosystems, University of Göttingen, Göttingen, Germany³University of Chinese Academy of Sciences, Beijing, PR China⁴Faculty of Geography, University of Marburg, Marburg, Germany⁵Institute of Environmental Sciences, Kazan Federal University, Kazan, Russia

Received 27 June 2016; Accepted 27 June 2016

ABSTRACT

A widespread pattern of the Tibetan plateau is mosaics of grasslands of *Cyperaceae* and grasses with forbs, interspersed with patches covered by lichen crusts induced by overgrazing. However, the fate of inorganic and organic N in non-crusted and crusted patches in *Kobresia* grasslands remains unknown. We reported on a field ¹⁵N-labeling experiment in two contrasting patches to compare retention of organic and inorganic N over a period of 29 days. ¹⁵N as KNO₃, (NH₄)₂SO₄ or glycine was sprayed onto soil surface. Crusted patches decreased plant and soil N stocks. More ¹⁵N from three N forms was recovered in soil than plants in both patches 29 days after the labeling. In non-crusted patches, ¹⁵N recovery by the living roots was about two times higher than in crusted ones, mainly because of higher root biomass. Microorganisms in non-crusted patches were N-limited because of more living roots and competed strongly for N with roots. Inorganic N input to non-crusted patches could alleviate N limitation to plants and microorganisms, and leads to higher total ¹⁵N recovery (plant + soil) for inorganic N forms. Compared to non-crusted patches, microorganisms in crusted patches were more C-limited because of depletion of available C caused by less root exudation. Added glycine could activate microorganisms, together with the hydrophobicity of glycine and crusts, leading to higher ¹⁵N-glycine than inorganic N. We conclude that overgrazing-induced crusts in *Kobresia* grasslands changed the fate of inorganic and organic N, and lead to lower total recovery from inorganic N but higher from organic N. Copyright © 2016 John Wiley & Sons, Ltd.

KEY WORDS: Tibetan grasslands; *Kobresia* grasslands; pasture degradation; crusted patches; ¹⁵N pulse labeling

INTRODUCTION

In most terrestrial ecosystems, N is often the most important nutrient limiting plant growth (LeBauer & Treseder, 2008). In the past 100 years, anthropogenic activities have greatly altered the terrestrial N cycling through a number of approaches. First, a large amount of N input has been occurred through biological N fixation of legumes (Herridge *et al.*, 2008), N fertilization (Vitousek *et al.*, 1997; Fernández-Sanjurjo *et al.*, 2014) and additions of plant residues and manure (Abbasi *et al.*, 2015) as well as rock weathering (Houlton & Morford, 2015). Second, soil redistribution and land cover caused by human activities also contribute to changes in the N stocks (Li *et al.*, 2014; Yu *et al.*, 2014; Hu *et al.*, 2015). Meanwhile, land use change also alters the N stocks through affecting N mineralization, soil erosion and structure (Campos *et al.*, 2014; Lozano-García & Parras-Alcántara, 2014; Yu & Jia, 2014; Gelaw *et al.*, 2015; Gümüs & Seker, 2015). Additionally, vegetation play an important role in the maintenance of soil functions, for example carbon input in the rhizosphere can conduce to

reclamation of mine spoil (Mukhopadhyay *et al.*, 2016) and plant residues can effectively reduce soil erosion and increase carbon sequestration (García-Díaz *et al.*, 2016; Prosdocimi *et al.*, 2016). Removal of vegetation caused by human activities can affect the soil N cycling (Wang *et al.*, 2005; Rutherford *et al.*, 2014) through reducing N input to soil and increasing erosion. As a result, plant growth will be further affected by such changes. This indicates that the vegetation and the soil system are very important to control the material and energy cycles and offer services to the humankind. The knowledge about the fate of added N in the vegetation and the soil system will conduce to the sustainability (Keesstra *et al.*, 2016), especially for those grasslands which serve as the pasture, where grazing caused by herbivores is an important land use type. Grazing often exerts strong effects on the vegetation and the soil system, respectively (Chapin *et al.*, 2000), for example increasing available N through urine and feces (Frank & Evans, 1997; Gao *et al.*, 2008), soil compaction by trampling (Cao *et al.*, 2004) and removal of aboveground parts (Wang *et al.*, 2008) and species replacement (Anderson & Briske, 1995).

About 48% of the Tibetan Plateau, the highest plateau on Earth with average altitude above 4000 m.a.s.l. and an area of 2.5 million km² (Lu *et al.*, 2004), is occupied by alpine grasslands (Sun & Zheng, 1998). These alpine grasslands

*Correspondence to: X. Xu, Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources, Chinese Academy of Sciences, Beijing, PR China.
E-mail: xuxingl@hotmail.com

store very large amounts of soil C and N and provide important ecosystem services such as livestock production and C sinks (Wang *et al.*, 2002; Yang *et al.*, 2008). However, these grasslands are mainly N limited because of slow mineralization of soil organic matter at the prevailing low temperatures (Song *et al.*, 2007; Gao *et al.*, 2014). Additionally, grazing is the most use in these grasslands. In the last 30 years, livestock numbers on the Tibetan Plateau have increased dramatically because of the government rangeland policy. As a result, overgrazing is probably the most important factor causing degradation of alpine grasslands through altering vegetation and the soil system (Zhou *et al.*, 2005; Lin *et al.*, 2015). Because of grazing, approximately 450,000 km² of the Tibetan grasslands is occupied by *Kobresia pygmaea* grasslands which are characterized by poor recovery ability after being destroyed (Miehe *et al.*, 2008, 2009; Unteregelsbacher *et al.*, 2012). The sedge *K. pygmaea* is well adapted to a moderate grazing intensity because of its specific plant traits, and thus it becomes the dominant species in this pastoral ecosystem (Miehe *et al.* 2008; He & Richards, 2015). For instance, *K. pygmaea* (i) forms thick root mats protecting against trampling by sheep and yaks (Cao *et al.*, 2004; Kaiser *et al.*, 2008; Miehe *et al.*, 2008; Unteregelsbacher *et al.*, 2012) and (ii) is very efficient for nutrient uptake because of its dense root network (Schleuss *et al.*, 2015).

Such large area of *K. pygmaea* grasslands is mainly ascribed to overgrazing, which has changed the vegetation and the soil system of alpine grasslands, for example causing species replacement (Rutherford *et al.*, 2014; Peng *et al.*, 2015) and affecting the C and N cycling (Wang *et al.*, 2005; Qiao *et al.*, 2015). The reason is that increasing grazing intensity reduces seedling emergence (Wang *et al.*, 2014), and disturbs reproduction of numerous grasses as well as changing soil physicochemical properties. Consequently, this stimulates the occupation

of patches by blue–green algae and crustose-lichen crusts (Eldridge, 2000; Unteregelsbacher *et al.*, 2012; He & Richards, 2015). Such crusts induced by overgrazing have been clearly observed in *Kobresia* grasslands on the Tibetan Plateau (Unteregelsbacher *et al.*, 2012; He & Richards, 2015). Clarifying how overgrazing-induced crusts affects the fate of added N can improve our understanding effects of overgrazing on the N cycling in alpine grasslands and thus conduce to management of alpine grasslands.

Additionally, a large number of studies have demonstrated that biological soil crusts can increase N input into soil and restore soil fertility as they can fix atmospheric N₂ in various terrestrial ecosystems globally (West, 1990; Belnap *et al.*, 2003; Zhao *et al.*, 2010). In arid and semi-arid grasslands, the existence of biological soil crusts can resist to erosion because filamentous cyanobacteria exuding polysaccharides that contribute to formation of soil aggregates (Aguilar *et al.*, 2009) and increase cohesion (Zhao *et al.*, 2006). On the Tibetan Plateau, biological soil crusts have also been confirmed to have benefits in non-degraded and lightly degraded grasslands, but its composition shifted with degradation processes, with some crusts being degraded and becoming black patches appeared in overgrazed alpine grasslands (Figure 1, Li *et al.*, 2015). Such crusts have modified various soil processes, for example they decrease infiltration rates into soil, and thus slow down decomposition of soil organic matter. Nutrient and water limitations further lead to more dead roots and lower microbial abundance and activity (Cao *et al.*, 2004; Babel *et al.*, 2014), thus modifying N mineralization and mobilization (Wang *et al.*, 2008; Cao & Long, 2009). This indicates that such crusts induced by overgrazing have detrimental effects on the material and energy cycles in alpine grasslands. Clarifying its effect on the fate of inorganic and organic N in alpine



Figure 1. Picture showed our study sites with two contrasting patches of vegetation in 07-2009. Left: Vegetation cover approximately 100% with predominant *Kobresia* and *Stipa* species (referred to as non-crusted patches). Right: With the same species, cover less than 30%, more than 70% of the surface is covered by blue–green algae and crustose-lichens (referred to as crusted patches). The bars on the scale are 10 cm. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr

grasslands could provide more insights into the role of biological soil crusts.

In Xinghai County on Tibetan Plateau, nearly 20% of the surface has been occupied by such crust patches (Figure 1), but few studies have been conducted to evaluate how overgrazing-induced crusts affect the fate of N in alpine grasslands. To clarify this effect, we sprayed ^{15}N -labeled glycine, NH_4^+ and NO_3^- to *Kobresia* grasslands with 100% vegetation cover (referred to as non-crust patches) and the other with the same species covering less than 30% of the soil (referred to as crusted patches) to trace ^{15}N in plant and soil components. We hypothesized that overgrazing-induced crusted patches held lower C and N stocks in the plant–soil system following decomposition and leaching processes (H1). Further, partitioning of glycine, NH_4^+ and NO_3^- in the plant–soil system should be different because of their distinct properties in soil solution, and overgrazing-induced crusted patches have lower N partitioning into plants (H2). Thus, total ^{15}N recovery from organic N is expected to be higher than from inorganic N in overgrazing-induced crusted patches because its microorganisms are more C limited than in non-crust patches (H3).

MATERIALS AND METHODS

Site Description

This study was conducted in Xinghai County of Qinghai province ($35^\circ32'\text{N}$, $99^\circ51'\text{E}$), on a loess-covered gravel terrace of the Huang He catchment at 3417 m a.s.l. in the northeastern part of the Tibetan Plateau. *K. humilis*/*K. pygmaea*-*Stipa* grasslands are dominant grassland types at the experimental site and are used as a winter pasture for yaks and sheep. The soils are classified as Haplic Kastanozems with a felty turf termed “*Kobresia* turf” in the upper horizon (Table I, Kaiser *et al.*, 2008; Miehe *et al.*, 2008; Unteregelsbacher *et al.*, 2012).

Annual precipitation (Xinghai climate station) averaged 353 mm, ranging from 214 to 483 mm in the 40 years from 1961 to 2001. Because of the East Asian monsoon, rains are mainly concentrated in summer. The rain gauge nearest to the study site recorded 448 mm a^{-1} , while the annual temperature ranged from 1°C to 5°C (2002–2007). Monthly temperatures between May and September exceed 5°C , and the average temperature from June to August was above 10°C (Miehe *et al.*, 2008). However, from December to January, the monthly temperature was lower than -10°C , and the minimal temperature was -33.5°C (Miehe *et al.*, 2008).

Table I. Properties of Haplic Kastanozem on the Tibetan Plateau near Xinghai (from Unteregelsbacher *et al.*, 2012). The values were mean ± 1 SE of four replicates

Depth (cm)	Horizon symbol	Soil structure	Bulk density (g cm^{-3})	pH	Corg (%)	Total N (%)	C/N ratio
0–6	Afe	Felty	0.60 ± 0.01	6.64 ± 0.15	8.80 ± 0.20	0.68 ± 0.01	12.9 ± 0.60
6–25	Ahk	Granular	0.81 ± 0.01	6.96 ± 0.19	4.38 ± 0.15	0.46 ± 0.01	9.52 ± 0.54
25–90	Bkc	Angular	0.84 ± 0.01	7.62 ± 0.04	2.28 ± 0.19	0.25 ± 0.02	9.12 ± 1.62

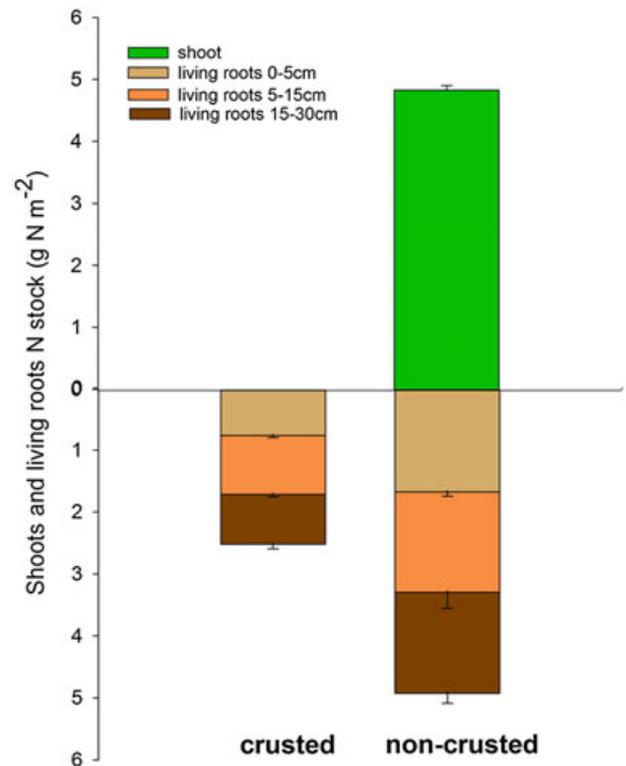


Figure 2. N stocks in shoots and living roots (g N m^{-2}) in crusted and non-crust patches. The values were mean ± 1 SE of four replicates. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr

^{15}N Labeling and Sampling

In this typical *Kobresia* grassland, a plot with an area of $30 \text{ m} \times 30 \text{ m}$ was selected for this study in July 2009. Twenty-four quadrats ($0.3 \text{ m} \times 0.3 \text{ m}$) were randomly selected in two contrasting patches: (i) vegetation cover approximately 100% with predominant *Kobresia* and *Stipa* species (referred to as non-crust patches), and (ii) the same species covering less than 30% of the soil, and more than 70% of the surface is covered by blue–green algae and crustose-lichens (referred to as crusted patches, Figure 1). The distance was at least 3 m between these quadrats. The quadrats were labeled with KNO_3 (60 atom% ^{15}N enrichment), $(\text{NH}_4)_2\text{SO}_4$ (60 atom% ^{15}N enrichment) or ^{15}N -glycine (99 atom% ^{15}N enrichment), respectively. Glycine, NH_4^+ and NO_3^- were used because they are present as dominant N forms for *Kobresia* grasslands (Xu *et al.*, 2011). Each treatment had four replicate quadrats.

^{15}N labeled substances were dissolved in 150-ml water and uniformly sprayed onto the plots with a small watering can. Additional distilled water (300 ml) was then slowly sprayed

Table II. Soil characteristics under crusted patches covered by lichen crusts and non-crusted patches with *Kobresia*. The values are mean \pm 1 SE of four replicates

	Non-crusted patches			Crusted patches		
	C stock* (g m ⁻²)	N stock (g m ⁻²)	C/N	C stock* (g m ⁻²)	N stock (g m ⁻²)	C/N
0–5 cm	2780 \pm 94b	232 \pm 8.9b	12.2 \pm 0.3a	2380 \pm 97a	175 \pm 6.6a	13.7 \pm 0.3b
5–15 cm	3405 \pm 172	353 \pm 70	9.6 \pm 0.3	3444 \pm 215	360 \pm 51	9.6 \pm 0.2
15–30 cm	2532 \pm 195	286 \pm 18	8.7 \pm 0.1	2896 \pm 386	304 \pm 81	9.1 \pm 0.2

*Unteregelsbacher *et al.* (2012).

onto each plot to ensure that no ¹⁵N remained on the plant leaves. The total N addition rate was 54 mg N m⁻² for each treatment. Shoots (only for non-crusted patches), living roots and soils were sampled at 1, 9, 18 and 29 days after ¹⁵N labeling from the same plot. Every time, shoots were clipped close to the soil surface with scissors within a quadrat (5 cm \times 5 cm) randomly in the same plot. Soils and roots were collected at different soil depths (0–5, 5–15 and 15–30 cm) using a soil corer (2.1-cm diameter) in each plot. Soil was sieved (2 mm) and roots were classified as dead and living based on their color and consistence. Additional five replicates were collected from unlabeled samples to measure isotope natural abundance.

All shoots, roots and soil samples were oven-dried at 60 °C and subsequently milled. Soil samples were treated with 5% HCl to remove carbonates and frozen dry. Total OC, N content and ¹⁵N/¹⁴N were measured using an isotope ratio mass spectrometer (Delta plus, Conflo III, Thermo Electron Cooperation, Bremen, Germany) coupled to an elemental analyzer (NA1500, Fisons instruments, Milano, Italy).

Calculation and Statistics

The plant N and C stocks (g m⁻²) were calculated by multiplying N or C contents by plant dry mass (g m⁻²). Soil N and C stocks (g m⁻²) in layers at 0–5 cm, 5–15 cm and 15–30 cm depth were calculated using the following equation:

$$\text{C or N stock} = c * z * \rho / 10^4 \quad (1)$$

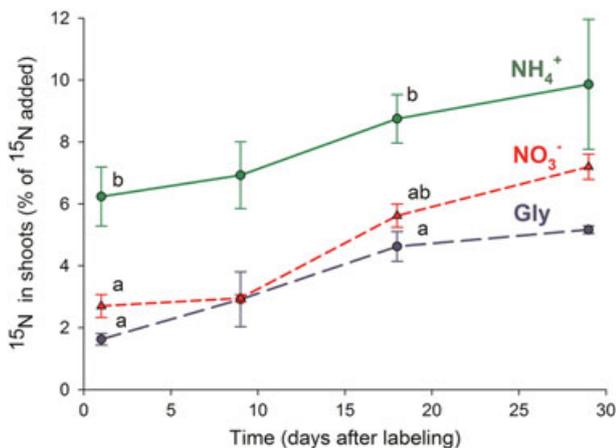


Figure 3. ¹⁵N uptake by *Kobresia pygmaea* shoots after ¹⁵N addition. The values were mean \pm 1 SE of four replicates. Different small letters at each sampling time point indicate significant difference between the three nitrogen forms (NH₄⁺, NO₃⁻ and glycine). This figure is available in colour online at wileyonlinelibrary.com/journal/ldr

Where *c* is the C or N content (g g⁻¹ soil), *z* (cm) is the thickness of the considered soil layer and ρ (g cm⁻³) is the bulk density.

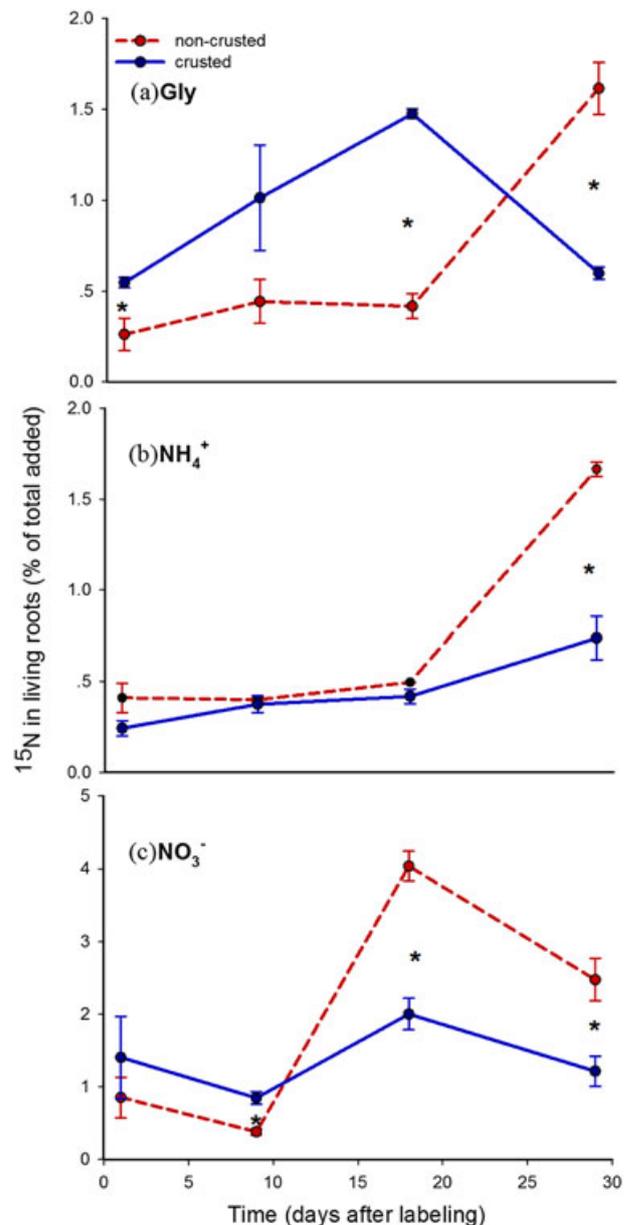


Figure 4. ¹⁵N recovery (\pm 1 SE) in living roots after ¹⁵N addition depending on N form (glycine, NH₄⁺ and NO₃⁻) and the presence of crusts. The values were mean \pm 1 SE of four replicates. Asterisk at each sampling time point indicates significant difference between crusted patches and non-crusted patches. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr

$^{15}\text{N}_{\text{upt}}$ ($\text{g } ^{15}\text{N m}^{-2}$) is the amount of ^{15}N recovered in different N pools (i.e. soil, shoots and roots), and was calculated as follows:

$$^{15}\text{N}_{\text{upt}} = \text{N}\% * \text{APE} * d \quad (2)$$

$$\text{APE} = ^{15}\text{N}_{\text{sample atom}\%} - ^{15}\text{N}_{\text{natural atom}\%} \quad (3)$$

Where N% is the sample N content, APE is the ^{15}N atom% excess (the difference of in ^{15}N atom% between labeled and unlabeled treatments) and d (g m^{-2}) is the dry matter pool sizes of soil, shoots or roots.

^{15}N recovery (% of added ^{15}N) was calculated according to the following equation:

$$^{15}\text{N}_{\text{rec}} = ^{15}\text{N}_{\text{upt}} / ^{15}\text{N}_{\text{input}} * 100\% \quad (4)$$

Where $^{15}\text{N}_{\text{rec}}$ is ^{15}N recovery (%), the percentage of ^{15}N recovered in soil or plants. $^{15}\text{N}_{\text{input}}$ ($\text{g } ^{15}\text{N m}^{-2}$) is the amount of ^{15}N added into the soil. $^{15}\text{N}_{\text{upt}}$ ($\text{g } ^{15}\text{N m}^{-2}$) is the amount of ^{15}N recovered in different N pools (i.e. soil, shoots and roots)

An independent-sample t -test was used to compare N stocks and C/N ratios under crusted and non-crusted patches. A three-way repeated ANOVA was used to compare the effects of degradation and the effects of the three N forms (glycine, NH_4^+ and NO_3^-) on ^{15}N recovery in soil and living roots at each sampling time (Table S1). All statistical analyses were performed after checking for normality and homogeneity of variances and were carried out with SPSS 16-0 (SPSS Inc., 200 Chicago, IL, USA). They were deemed significant at $P < 0.05$.

RESULTS

Above- and Belowground Biomass and N Stocks

In non-crusted patches, the aboveground biomass was $226 \pm 18.4 \text{ g m}^{-2}$. The living roots biomass was $267 \pm 36.5 \text{ g m}^{-2}$ at 0–5 cm layer, with a deeper soil layers (5–15 and 15–30 cm). Crusted patches living roots biomass was about two times lower than in non-crusted patches. Correspondingly, the N stocks in shoots, living roots and the upper (0–5 cm) soil were higher in non-crusted patches than in crusted patches (Figure 2; Table II). However, there was no difference in the soil N stocks of 5–15 cm and 15–30 cm

layers between non-crusted and crusted patches. In the upper 5 cm, crusted patches showed significant higher soil C/N than the non-crusted patches (Table II).

^{15}N Uptake by Plants

^{15}N recovered in shoots was the highest for NH_4^+ but the lowest for glycine in non-crusted patches (Figure 3). ^{15}N uptake in shoots increased with time as more ^{15}N was taken up and transferred from roots to shoots for all N forms.

^{15}N -Glycine recovery in living roots was higher in crusted than in non-crusted patches until the 18th day. It steeply decreased in crusted patches, whereas it still increased with time in non-crusted patches (Figure 4a). ^{15}N - NH_4^+ recovery in living roots increased with time in both patches (Figure 4b). The recovery of ^{15}N from NO_3^- was different between both patches at 9th, 18th and 29th days after the labeling (Figure 4c). on the 29th day, ^{15}N recovery in living roots was two times higher for all three N forms under non-crusted than under crusted patches (Table III, Figure 4).

^{15}N Remaining in Soil

^{15}N remaining in soil of crusted and non-crusted patches showed a similar trend for NH_4^+ and NO_3^- , but significantly differ on day 1 and day 9. ^{15}N recovery in soil decreased with time (except for $^{15}\text{NO}_3^-$ on day 9, Figure 5b, c). ^{15}N - NH_4^+ soil recovery was similar with ^{15}N - NO_3^- in both patches. In contrast, ^{15}N -glycine recovery showed an opposite pattern than both inorganic N forms: more ^{15}N was recovered in crusted patches (Figure 5a).

^{15}N Recovery on the 29th Day After Labeling

Total ^{15}N recovery from glycine in non-crusted patches were significantly higher than crusted patches, while ^{15}N recovery of NH_4^+ and NO_3^- in non-crusted patches were significantly lower (Table III). Most of ^{15}N was recovered in soil in all treatments. ^{15}N recovery from shoots was not significantly different among three N forms in non-crusted patches (Table III). Total ^{15}N - NH_4^+ soil recovery was similar with ^{15}N - NO_3^- in both patches. Among three N forms, glycine showed highest total ^{15}N recovery in crusted patches, but lowest in non-crusted patches (Table III).

Table III. ^{15}N recovery from three N forms (glycine, NH_4^+ and NO_3^-) for various N pools in Tibetan grassland 29 days after labeling (% of total added). The values were mean \pm 1 SE of four replicates. "a" presents the ^{15}N recovery difference between crusted patches and non-crusted patches, "A" presents the difference among NH_4^+ , glycine and NO_3^-

^{15}N recovery (%)	Glycine		NH_4^+		NO_3^-	
	Non-crusted patches	Crusted patches	Non-crusted patches	Crusted patches	Non-crusted patches	Crusted patches
Soil (0–5 cm)	20.6 \pm 4.8 ^a _A	62.4 \pm 7.1 ^b _B	37.3 \pm 2.6 _B	36.0 \pm 1.5 _A	43.4 \pm 1.8 _B	39.0 \pm 2.0 _A
Above ground (shoots)	5.2 \pm 0.1		9.9 \pm 2.1		7.2 \pm 0.4	
Living roots	1.6 \pm 0.1 ^b _A	0.6 \pm 0.03 ^a _A	1.7 \pm 0.04 ^b _{AB}	0.7 \pm 0.1 ^a _{AB}	2.5 \pm 0.3 ^b _B	1.2 \pm 0.2 ^a _B
Total recovery	27.4 \pm 4.8 ^a _A	63.0 \pm 7.1 ^b _B	48.8 \pm 3.3 ^b _B	36.7 \pm 1.5 ^a _A	53.1 \pm 1.9 ^b _B	40.2 \pm 2.0 ^a _A

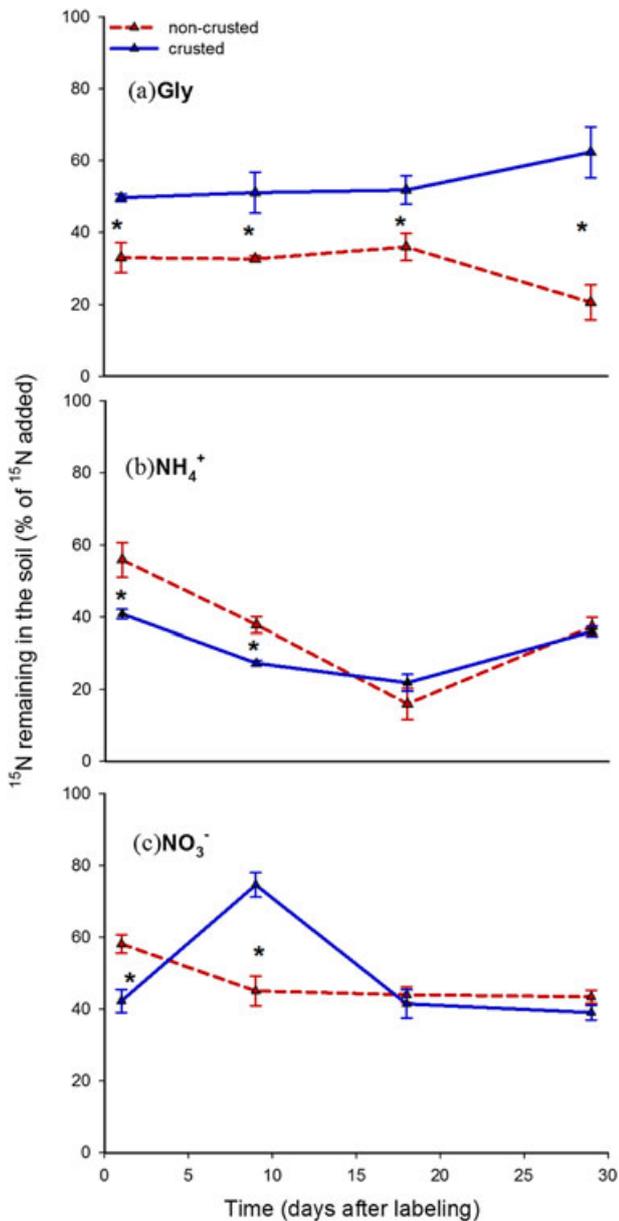


Figure 5. Dynamics of ^{15}N (± 1 SE) remaining in soil after ^{15}N addition, depending on N form (glycine, NH_4^+ , NO_3^-) and the presence of crust. The values were mean ± 1 SE of four replicates. Asterisk at each sampling time point indicates significant difference between crusted patches and non-crusted patches. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr

DISCUSSION

Mosaics of felty root mats, with crusts covered by blue-green algae and crustose-lichens, and *Kobresia* grasslands are a widespread and specific pattern of the pastoral ecosystem in the northeastern quarter of the Tibetan Plateau. The C and N stocks in crusted patches were lower than in non-crusted patches only in the 0–5 cm soil layer (Table II). This could be ascribed to that strong trampling of the upper soil imposed by sheep and yaks increases decomposition (Cao *et al.*, 2004) and removal of aboveground parts reduced litter return (Peng *et al.*, 2015). Besides, more N loss through nitrate leaching, ammonia (NH_3) volatilization or nitrous

oxide emissions could decrease soil N content in the surface soil layer of crusted patches during degradation (Butterbach-Bahl *et al.*, 2011).

Shoots accumulated more ^{15}N with time (Figure 3). This may be explained by a continuous ^{15}N uptake and transfer from roots to shoots during long-term periods (Kuzyakov & Xu, 2013). At 29 days after the labeling, living roots in non-crusted patches recovered two times more ^{15}N than in crusted patches, which is consistent with living roots biomass difference.

Previous studies showed that ^{15}N recovery from NH_4^+ and NO_3^- was different because of their distinct properties (Xu *et al.*, 2003, 2004). We confirmed this within 18 days after the labeling, for example ^{15}N recovery by plants was higher from NH_4^+ than from NO_3^- in the first 9 days. This could be ascribed to the preferential uptake of NH_4^+ by plants. Total ^{15}N recovery was significantly lower from NH_4^+ than from NO_3^- during the period from 9 days to 18 days perhaps because NH_3 volatilization caused by high pH (Cameron *et al.*, 2013). Total ^{15}N recovery from both N forms remained similarly in crusted or non-crusted patches at 29 days after labeling (Figure 5 b, c). Possible explanation is rapid transformation from NH_4^+ to NO_3^- could lead to no difference in ^{15}N for NH_4^+ and NO_3^- in soil solution, and resulted in similar ^{15}N recovery over a long-term period. Higher ^{15}N recovery in soil from NH_4^+ and NO_3^- in non-crusted patches than in crusted patches in the first day after labeling could be ascribed to more N limitation to soil microorganisms in non-crusted patches. In non-crusted patches, more living roots could produce more root exudates and result in higher C availability and enhance microbial growth (Blagodatskaya & Kuzyakov, 2008). Therefore, soil microbes require more N, but need further investigations. After 9 days after labeling, $^{15}\text{NO}_3^-$ recovery in crusted patches was two times higher than in non-crusted patches. This could be the result of crusts which released $^{15}\text{NO}_3^-$ they recovered in the initial period.

In contrast to inorganic N, higher ^{15}N recovery from glycine in crusted patches reflected that more C limited because of less living roots. Most microorganisms are inactive because of limitation of C and energy if there is no available C input (Song *et al.*, 2010; Blagodatskaya & Kuzyakov, 2013). Addition of traced C to soil can induce a small but significant increase on microbial biomass through triggering effect (De Nobili *et al.*, 2001). Here we added a small amount of glycine in C-limited crusted patches, which could stimulate microbial activity. This conduces to higher glycine ^{15}N retention than inorganic N in crusted patches. Another reason was that less glycine-N is leached to deeper soil because of glycine may be well fixed in soil because of glycine hydrophobicity. Additionally, crusts are water repellent and contribute to delayed infiltration (Eldridge, 2000; Kröpfl *et al.*, 2013; He & Richards, 2015). Overgrazing-induced crusted patches covered with crusts without vegetation are exposed to solar irradiation. Solar irradiation will heat up the crust upper soil, leading to much higher evaporation,

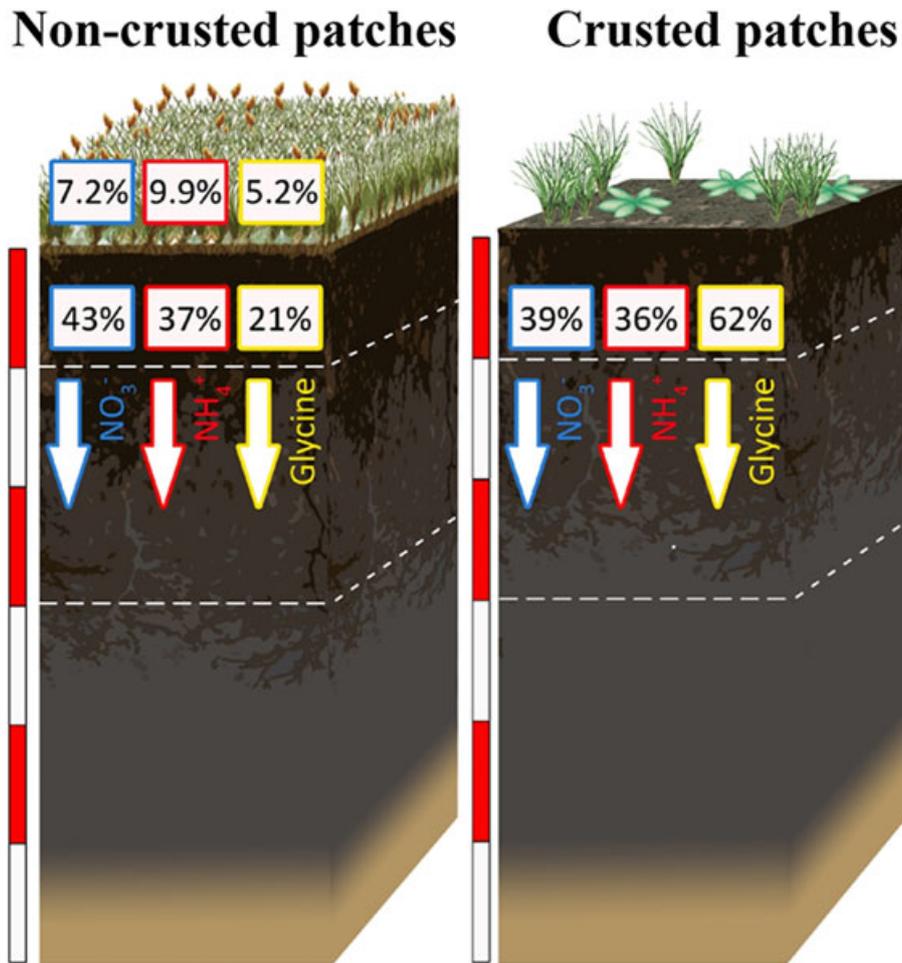


Figure 6. A summary for the fate of inorganic and organic nitrogen in both non-crust and crusted patches in a *Kobresia* grassland. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr

while *Kobresia* root mats were extremely well insulated (Cerdà, 1997; Unteregelsbacher *et al.*, 2012). As a result, the water penetration under crusts is much slower than in non-crust patches. Additionally, higher temperatures boost decomposition in topsoil (Kuziyakov *et al.*, 2007; Heitkamp *et al.*, 2012). This supports our third hypothesis that crusted patches recovered more ¹⁵N-glycine. Total ¹⁵N recovery from NH₄⁺ and NO₃⁻ in non-crust pasture was about 50%, higher than glycine, suggesting that crusted patches covered by crusts changed the fate of inorganic and organic N (Figure 6).

CONCLUSIONS

Patches covered by blue-green algae and crustose-lichen crusts which are induced by overgrazing, are often observed in *Kobresia* grasslands. Such crusts have changed the N cycling in alpine grasslands. In the crusted patches, crusts decreased plant and soil N stocks. At 29 days after labeling, ¹⁵N recovery by living roots in non-crust patches was about two times higher than in crusted patches because of higher root biomass. Higher total inorganic ¹⁵N in non-crust patches than crusted patches could be because

of more N limitation. Microorganisms could be more competitive, when they were exposed to high living root biomass and more root exudates. By comparison, higher ¹⁵N-glycine recovery than inorganic N in crusted patches can be explained by the fact that microorganisms were more C limited because of a lack of root exudates, and glycine addition increased microbial activity. Additional explanations are the hydrophobicity of glycine and crusts. Finally, we conclude that degradation of *Kobresia* grasslands on the Tibetan Plateau changed the fate of inorganic and organic N, and lead to lower total recovery from inorganic N but higher from organic N.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (31470560), and the German Research Council Priority Programme 1372 “Tibetan Plateau – Formation – Climate – Ecosystems (TiP)” with the contracts KU 1184/14-1,2. We thank the Centre for Stable Isotope Research and Analysis (KOSI) of Göttingen for ¹⁵N isotope analysis.

REFERENCES

- Abbasi MK, Tahir MM, Sabir N, Khurshid M. 2015. Impact of the addition of different plant residues on nitrogen mineralization-immobilization turnover and carbon content of a soil incubated under laboratory conditions. *Solid Earth* **6**: 197–205. DOI:10.5194/se-6-197-2015.
- Aguilar AJ, Huber-Sannwald E, Belnap J, Smart DR, Moreno JA. 2009. Biological soil crusts exhibit a dynamic response to seasonal rain and release from grazing with implications for soil stability. *Journal of Arid Environments* **73**: 1158–1169. DOI:10.1016/j.jaridenv.2009.05.009.
- Anderson VJ, Briske DD. 1995. Herbivore-induced species replacement in grasslands: is it driven by herbivory tolerance or avoidance? *Ecological Applications* **5**: 1014–1024. DOI:10.2307/2269351.
- Babel W, Biermann T, Coners H, Falge E, Seeber E, Ingrisch J, Schleuß PM, Gerken T, Leonbacher J, Leopold T, Willinghöfer S, Schützenmeister K, Shibistova O, Becker L, Hafner S, Spielvogel S, Li X, Xu X, Sun Y, Zhang L, Yang Y, Ma Y, Wesche K, Graf HF, Leuschner C, Guggenberger G, Kuzyakov Y, Miede G, Foken T. 2014. Pasture degradation modifies the water and carbon cycles of the Tibetan highlands. *Biogeosciences* **11**: 6633–6656. DOI:10.5194/bg-11-6633-2014.
- Belnap J, Büdel B, Lange OL. 2003. *Biological soil crusts: characteristics and distribution*. Springer Berlin: Heidelberg; 3–30.
- 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. DOI: 10.1007/s00374-008-0334-y
- Blagodatskaya E, Kuzyakov Y. 2013. Active microorganisms in soil: critical review of estimation criteria and approaches. *Soil Biology and Biochemistry* **67**: 192–211. DOI:10.1016/j.soilbio.2013.08.024.
- Butterbach-Bahl K, Gundersen P, Ambus P, Augustin J, Beier C, Boeckx P, et al. 2011. Nitrogen processes in terrestrial ecosystems. In *The European nitrogen assessment: sources, effects and policy perspectives*. Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Grennfelt P, et al. (eds.). Cambridge University Press: Cambridge, UK; 99–125.
- Cameron KC, Di HJ, Moir JL. 2013. Nitrogen losses from the soil/plant system: a review. *Annals of Applied Biology* **162**: 145–173. DOI:10.1111/aab.12014.
- Campos AC, Etchevers JB, Oleschko KL, Hidalgo CM. 2014. Soil microbial biomass and nitrogen mineralization rates along an altitudinal gradient on the cofre de perote volcano (Mexico): the importance of landscape position and land use. *Land Degradation & Development* **25**: 581–593. DOI:10.1002/ldr.2185.
- Cao GM, Long RJ. 2009. System stability and its self-maintaining mechanism by grazing in Alpine *Kobresia* meadow. *Chinese Journal of Agrometeorology* **30**: 553–559.
- Cao GM, Lin L, Zhang F, Li Y, Han D, Long R. 2004. A review of maintenance, loss and recovery of stability of alpine *Kobresia humilis* meadow on Tibetan Plateau. *Pracultural Science* **27**: 34–38.
- Cerdà A. 1997. The effect of patchy distribution of *Stipa tenacissima* L. on runoff and erosion. *Journal of Arid Environments* **36**: 37–51. DOI:10.1006/jare.1995.0198.
- Chapin FS, III, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE, Mack MC, Díaz S. 2000. Consequences of changing biodiversity. *Nature* **405**: 234–242. DOI:10.1038/35012241.
- De Nobili M, Contin M, Mondini C, Brookes PC. 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate. *Soil Biology & Biochemistry* **33**: 1163–1170. DOI:10.1016/S0038-0717(01)00020-7.
- Eldridge D. 2000. Ecology and management of biological soil crusts: recent developments and future challenges. *The Bryologist* **103**: 742–747. DOI:10.1639/0007-2745(2000)103[0742:EAMOBS]2.0.CO;2.
- Fernández-Sanjurjo MJ, Alvarez-Rodríguez E, Núñez-Delgado A, Fernández-Marcos ML, Romar-Gasalla A. 2014. Nitrogen, phosphorus, potassium, calcium and magnesium release from two compressed fertilizers: column experiments. *Solid Earth* **5**: 1351–1360. DOI:10.5194/se-5-1351-2014.
- Frank DA, Evans RD. 1997. Effects of native grazers on grassland N cycling in Yellowstone National Park. *Ecology* **78**: 2238–2248. DOI:10.1890/0012-9658(1997)078.
- Gao YH, Luo P, Wu N, Chen H, Wang GX. 2008. Impacts of grazing intensity of nitrogen pools and nitrogen cycle in an alpine meadow of the eastern Tibetan plateau. *Applied Ecology and Environmental Research* **6**: 69–79.
- Gao JQ, Mo Y, Xu XL, Zhang XW, Yu FH. 2014. Spatiotemporal variations affect uptake of inorganic and organic nitrogen by dominant plant species in an alpine wetland. *Plant and Soil* **381**: 271–278. DOI:10.1007/s11104-014-2130-9.
- García-Díaz A, Bienes-Allas R, Gristina L, Cerdà A, Novara A, Pereira P. 2016. Carbon input threshold for soil carbon budget optimization in eroding vineyards. *Geoderma* **271**: 144–149. DOI:10.1016/j.geoderma.2016.02.020.
- Gelaw AM, Singh BR, Lal R. 2015. Organic carbon and nitrogen associated with soil aggregates and particle sizes under different land uses in Tigray, northern Ethiopia. *Land Degradation & Development* **26**: 690–700. DOI:10.1002/ldr.2261.
- Gümüş I, Şeker C. 2015. Influence of humic acid applications on modulus of rupture, aggregate stability, electrical conductivity, carbon and nitrogen content of a crusting problem soil. *Solid Earth* **6**: 1231–1236. DOI:10.5194/se-6-1231-2015.
- He S, Richards K. 2015. Impact of meadow degradation on soil water status and pasture management – a case study in Tibet. *Land Degradation & Development* **26**: 468–479. DOI:10.1002/ldr.2358.
- Heitkamp F, Jacobs A, Jungkunst HF, Heinze S, Wendland M, Kuzyakov Y. 2012. Processes of soil carbon dynamics and ecosystem carbon cycling in a changing world. In *Recarbonization of the biosphere*. Springer: Netherlands; 395–428. DOI: 10.1007/978-94-007-4159-1_18
- Herridge DF, Peoples MB, Boddey RM. 2008. Global inputs of biological nitrogen fixation in agricultural systems. *Plant and Soil* **311**: 1–18. DOI:10.1007/s11104-008-9668-3.
- Houlton BZ, Morford SL. 2015. A new synthesis for terrestrial nitrogen inputs. *The Soil* **1**: 381–397. DOI:10.5194/soil-1-381-2015.
- Hu YL, Niu ZX, Zeng DH, Wang CY. 2015. Soil amendment improves tree growth and soil carbon and nitrogen pools in Mongolian pine plantations on post-mining land in northeast China. *Land Degradation & Development* **26**: 807–812. DOI:10.1002/ldr.2386.
- Kaiser K, Miede G, Barthelmes A, Ehrmann O, Scharf A, Schult M, Schlütz F, Adamczyk S, Frenzel B. 2008. Turf-bearing topsoils on the central Tibetan Plateau, China: pedology, botany, geochronology. *Catena* **73**: 300–311. DOI:10.1016/j.catena.2007.12.001.
- Keesstra SD, Bouma J, Wallinga J, Tittone P, Smith P, Cerdà A, Montanarella L, Quinton JN, Pachepsky Y, van der Putten WH, Bardgett RD, Moolenaar S, Mol G, Jansen B, Fresco LO. 2016. The significance of soils and soil science towards realization of the United Nations Sustainable Development Goals. *The Soil* **2**: 111–128. DOI:10.5194/soil-2-111-2016.
- Kröppel AI, Cecchi GA, Villasuso NM, Distel RA. 2013. Degradation and recovery processes in semi-arid patchy rangelands of northern of Northern Patagonia, Argentina. *Land Degradation & Development* **24**: 393–399. DOI:10.1002/ldr.1145.
- Kuzyakov Y, Xu X. 2013. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. *New Phytologist* **198**: 656–669. DOI:10.1111/nph.12235.
- Kuzyakov Y, Hill PW, Jones DL. 2007. Root exudate components change litter decomposition in a simulated rhizosphere depending on temperature. *Plant and Soil* **290**: 293–305. DOI:10.1007/s11104-006-9162-8.
- LeBauer DS, Treseder KK. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**: 371–379. DOI:10.1890/06-2057.1.
- Li QY, Fang HY, Sun LY, Cai QG. 2014. Using the ¹³⁷Cs technique to study the effect of soil redistribution on soil organic carbon and total nitrogen stocks in an agricultural catchment of Northeast China. *Land Degradation & Development* **25**: 350–359. DOI:10.1002/ldr.2144.
- Li YK, Ouyang JZ, Lin L, Zhang FW, Du YG, Cao GM, Han F. 2015. Evolution characteristics of biological soil crusts (BSCs) during alpine meadow degradation. *Chinese Journal of Ecology* **34**: 2238–2244.
- Lin L, Li YK, Xu XL, Zhang FW, Du YG, Liu SL, Guo XW, Cao GM. 2015. Predicting parameters of degradation succession processes of Tibetan *Kobresia* grasslands. *Solid Earth* **6**: 1237–1246. DOI:10.5194/se-6-1237-2015.
- Lozano-García B, Parras-Alcántara L. 2014. Variation in soil organic carbon and nitrogen stocks along a toposequence in a traditional mediterranean olive grove. *Land Degradation & Development* **25**: 297–304. DOI:10.1002/ldr.2284.
- Lu HY, Wu NQ, Gu ZY, Guo ZT, Wang L, Wu HB, Wang G, Zhou LP, Han JM, Liu TS. 2004. Distribution of carbon isotope composition of modern soils on the Qinghai Tibetan Plateau. *Biogeochemistry* **70**: 273–297. DOI:10.1023/B:BIOG.0000049343.48087.ac.

- Miehe G, Miehe S, Kaiser K, Liu JQ, Zhao XQ. 2008. Status and dynamics of Kobresia pygmaea ecosystem on the Tibetan plateau. *Ambio* **37**: 272–279. DOI:10.1579/0044-7447(2008)37[272:SADOTK]2.0.CO;2.
- Miehe G, Miehe S, Kaiser K, Reudenbach C, Behrendes L, Duo L, Schlüt F. 2009. How old is pastoralism in Tibet? An ecological approach to the making of a Tibetan landscape. *Palaeogeography, Palaeoclimatology, Palaeoecology* **276**: 130–147. DOI:10.1016/j.palaeo.2009.03.005.
- Mukhopadhyay S, Mastro SE, Cerdà A, Ram LC. 2016. Rhizosphere soil indicators for carbon sequestration in a reclaimed coal mine spoil. *Catena* **141**: 100–108. DOI:10.1016/j.catena.2016.02.023.
- Peng F, Quangan Y, Xue X, Guo J, Wang T. 2015. Effects of rodent-induced land degradation on ecosystem carbon fluxes in an alpine meadow in the Qinghai–Tibet Plateau, China. *Solid Earth* **6**: 303–310. DOI:10.5194/se-6-303-2015.
- Prosdocimi M, Jordán A, Tarolli P, Keesstra S, Novara A, Cerdà A. 2016. The immediate effectiveness of barley straw mulch in reducing soil erodibility and surface runoff generation in Mediterranean vineyards. *Science of the Total Environment* **547**: 323–330. DOI:10.1016/j.scitotenv.2015.12.076.
- Qiao N, Xu X, Cao G, Ouyang H, Kuzyakov Y. 2015. Land use change decreases soil carbon stocks in Tibetan grasslands. *Plant and Soil* **395**: 231–241. DOI:10.1007/s11104-015-2556-8.
- Rutherford MC, Powrie LW, Husted LB. 2014. Herbivores-driven land degradation: consequences for plant diversity and soil in arid Subtropical Thicket in South-Eastern Africa. *Land Degradation & Development* **25**: 541–553. DOI:10.1002/ldr.2181.
- Schleuss PM, Heitkamp F, Sun Y, Miehe G, Xu X, Kuzyakov Y. 2015. Nitrogen uptake in an Alpine Kobresia pasture on the Tibetan Plateau: localization by ¹⁵N labeling and implications for a vulnerable ecosystem. *Ecosystems* **18**: 946–957. DOI:10.1007/s10021-015-9874-9.
- Song MH, Xu XL, Hu QW, Tian YQ, Ouyang H, Zhou CP. 2007. Interactions of plant species mediated plant competition for inorganic nitrogen with soil microorganisms in an alpine meadow. *Plant and Soil* **297**: 127–137. DOI:10.1007/s11104-007-9326-1.
- Song M, Jiang J, Cao G, Xu X. 2010. Effects of temperature, glucose and inorganic nitrogen inputs on carbon mineralization in a Tibetan alpine meadow soil. *European Journal of Soil Biology* **46**: 375–380. DOI:10.1016/j.ejsobi.2010.09.003.
- Sun HL, Zheng D. 1998. *Formation, evolution and development of Qinghai–Xizang (Tibetan) plateau*. Guangdong Science and Technology Press: Guangzhou; 1–10.
- Unteregelsbacher S, Hafner S, Guggenberger G, Miehe G, Xu X, Liu J, Kuzyakov Y. 2012. Response of long-, medium- and short-term processes of the carbon budget to overgrazing-induced crusts in the Tibetan Plateau. *Biogeochemistry* **111**: 187–201. DOI:10.1007/s10533-011-9632-9.
- Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* **7**: 737–750. DOI:10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2.
- Wang GX, Qian J, Cheng GD, Lai YM. 2002. Soil organic carbon pool of grassland soils on the Qinghai–Tibetan Plateau and its global implication. *Science of the Total Environment* **291**: 207–217. DOI:10.1016/S0048-9697(01)01100-7.
- Wang WY, Wang QJ, Wang CY, Shi HL, Li Y, Wang G. 2005. The effect of land management on carbon and nitrogen status in plants and soils of alpine meadows on the Tibetan plateau. *Land Degradation & Development* **16**: 405–415. DOI:10.1002/ldr.661.
- Wang CT, Wang QL, Jing ZC, Feng BF, Du YG, Long RJ, Cao GM. 2008. Vegetation roots and soil physical and chemical characteristic changes in *Kobresia pygmaea* meadow under different grazing gradients. *Acta Prataculturae Sinica* **5**: 9–15.
- Wang N, Jiao JY, Lei D, Chen Y, Wang DL. 2014. Effect of rainfall erosion: seedling damage and establishment problems. *Land Degradation & Development* **25**: 565–572. DOI:10.1002/ldr.2183.
- West NE. 1990. Structure and functions of microphytic soil crusts in wild-land ecosystems of arid to semi-arid regions. *Advances in Ecological Research* **20**: 179–223. DOI:10.1016/S0065-2504(08)60055-0.
- Xu XL, Ouyang H, Pei ZY, Zhou CP. 2003. Fate of ¹⁵N labeled nitrate and ammonium salts added to an alpine meadow in the Qinghai–Xizang Plateau, China. *Acta Botanica Sinica* **45**: 276–281.
- Xu XL, Ouyang H, Pei ZY, Zhou CP. 2004. Long-term partitioning of ammonium and nitrate among different components in an alpine meadow ecosystem. *Acta Botanica Sinica* **46**: 279–283.
- Xu X, Ouyang H, Cao G, Richter A, Wanek W, Kuzyakov Y. 2011. Dominant plant species shift their nitrogen uptake patterns in response to nutrient enrichment caused by a fungal fairy in an alpine meadow. *Plant and Soil* **341**: 495–504. DOI:10.1007/s11104-010-0662-1.
- Yang Y, Fang J, Tang Y, Ji C, Zheng C, He J, Zhu B. 2008. Storage, patterns and controls of soil organic carbon in the Tibetan grasslands. *Global Change Biology* **14**: 1592–1599. DOI:10.1111/j.1365-2486.2008.01591.x.
- Yu Y, Jia ZQ. 2014. Changes in soil organic carbon and nitrogen capacities of *Salix cheilophila* Schneid along a revegetation chronosequence in semi-arid degraded sandy land of the Gonghe Basin, Tibet Plateau. *Solid Earth* **5**: 1045. DOI:10.5194/se-5-1045-2014.
- Yu B, Stott P, Di XY, Yu HX. 2014. Assessment of land cover changes and their effect on soil organic carbon and soil total nitrogen in Daqing prefecture, China. *Land Degradation & Development* **25**: 520–531. DOI:10.1002/ldr.2169.
- Zhao YG, Xu MX, Wang QJ, Shao MA. 2006. Impact of biological soil crust on soil physical and chemical properties of rehabilitated grassland in hilly Loess Plateau, China. *Journal of Natural Resources* **21**: 441–448.
- Zhao YG, Xu MX, Belnap J. 2010. Potential nitrogen fixation activity of different aged biological soil crusts from rehabilitated grasslands of the hilly Loess Plateau, China. *Journal of Arid Environments* **74**: 1186–1191. DOI:10.1016/j.jaridenv.2010.04.006.
- Zhou H, Zhao X, Tang Y, Gu S, Zhou L. 2005. Alpine grassland degradation and its control in the source region of the Yangtze and Yellow Rivers, China. *Grassland Science* **51**: 191–203. DOI:10.1111/j.1744-697X.2005.00028.x.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web site.