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REGULAR ARTICLE

Nitrogen turnover and greenhouse gas emissions in a tropical alpine ecosystem, Mt. Kilimanjaro, Tanzania

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Abstract
Background and aims Tropical alpine ecosystems are identified as the most vulnerable to global environmental change, yet despite their sensitivity they are among the least studied ecosystems in the world. Despite its important role in constraining potential changes to the carbon balance, soil nitrogen (N) turnover and plant availability in high latitude and high altitude ecosystems is still poorly understood.
Methods Here we present a first time study on a tropical alpine *Helichrysum* ecosystem at Mt. Kilimanjaro, Tanzania, which lies at an altitude of 3880 m. Vegetation composition is characterized and major gross N turnover rates are investigated using the ^{15}N pool dilution method for three different vegetation cover types. In addition greenhouse gas exchange (CO_2, N_2O and CH_4) was manually measured using static chambers.
Results Gross N turnover rates and soil CO_2 and N_2O emissions were generally lower than values reported for temperate ecosystems, but similar to tundra ecosystems. Gross N mineralization, NH_4^+ immobilization rates, and CO_2 emissions were significantly higher on densely vegetated plots than on sparsely vegetated plots. Relative soil N retention was high and increased with vegetation cover, which suggests high competition for available soil N between microbes and plants. Due to high percolation rates, irrigation/rainfall has no impact on N turnover rates and greenhouse gas (GHG) emissions. While soil N_2O fluxes were below the detection limit at all plots, soil respiration rates and CH_4 uptake rates were higher at the more densely vegetated plots. Only soil respiration rates followed the pronounced diurnal course of air and soil temperature.
Conclusion Overall, our data show a tight N cycle dominated by closely coupled ammonification-NH_4^+-immobilization, which is little prone to N losses. Warming could enhance vegetation cover and thus N turnover; however, only narrower C:N ratios due to atmospheric nitrogen deposition may open the N cycle of *Helichrysum* ecosystems.

Keywords Soil N cycling · Gross N turnover · ^{15}N pool dilution · Greenhouse gas emission · Tropical alpine ecosystem

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Introduction

Due to harsh environmental conditions pushing organisms close to their physiological limits, high latitude and high altitude ecosystems are among the most vulnerable ecosystems affected by global environmental changes. Furthermore, these ecosystems are exposed to extraordinarily strong warming well above the global average (Wookey et al. 2009). Typically, productivity of these ecosystems is strongly limited by the availability of nitrogen (N) and phosphorus (P) (Shaver and Chapin 1991; Güsewell 2004; Weintraub and Schimel 2005). In a warming climate, the delicate balance of increased primary productivity (induced by higher nitrogen availability) and carbon (C) losses from intensified decomposition of SOM, may determine whether high latitude and high altitude ecosystems become a net sink or source for atmospheric carbon dioxide. Alternatively, the vegetation itself may exert a feedback on soil C and N cycling through its litter quality, root exudation of labile organic compounds and via competition for organic and mineral nutrients (Rennenberg et al. 2009; Chapman et al. 2006). Despite its important role in constraining potential changes to the C balance, soil N turnover and plant availability in high latitude and high altitude ecosystems are still poorly understood (Weintraub and Schimel 2005). In particular this holds for tropical alpine ecosystems, which are considered to be one of the least well investigated ecosystems in the world (Buytaert et al. 2011). To our knowledge the study of Schmidt et al. (2009) is currently the only soil biogeochemical study providing gross N turnover rates for a tropical alpine ecosystem exposed to extreme diurnal temperature fluctuation. Studies on biogeochemical nutrient cycling are more widely available for higher latitude and alpine ecosystems in the temperate zone (e.g., Jaeger et al. 1999; Ernakovich et al. 2014; Clein and Schimel 1995; Alm et al. 1999; Gulledge and Schimel 2000; Kielland et al. 2006; Kielland et al. 2007; Kurganova et al. 2003). However, environmental conditions in tropical alpine ecosystems at >4000 m are not directly comparable to those ecosystems due to generally lower atmospheric pressure, higher UV irradiance and different rainfall regimes. Even more, tropical alpine ecosystems are rather exposed to extreme diurnal temperature and radiation variations, whereas high latitude and alpine ecosystems are subject to strong seasonal variations of soil and air temperature as well as solar radiation resulting in highest activity of plant and biogeochemical soil processes in summer (Schmidt et al. 2009). Nevertheless, it was reported that even during periods of low soil temperatures (< 5 °C), and in particular during freeze-thaw events, microbes are still active and contribute to significant rates of gross soil N turnover (Schmidt et al. 2009; Wu et al. 2012; Wolf et al. 2010; Schütt et al. 2014) and associated N_2O emissions with significant or even dominating contribution to the annual budgets (Holst et al. 2008; Luo et al. 2012). Various physical, chemical and biological processes and their interactions are proposed to explain the occurrence of low temperature related N_2O emissions (De Bruijn et al. 2009; Matzner and Borken 2008). Due to pronounced diurnal changes in air and soil temperature freeze-thaw events could occur in tropical alpine ecosystems at unprecedented temporal frequency and are likely disruptive to soil microbial communities with hitherto unresolved impacts on ecosystem availability of soil N (Larsen et al. 2002; Henry 2007).

Therefore, for the first time, we conducted a field study in an African *Helichrysum* ecosystem, with the aim of improving our understanding of soil N cycling and availability in a tropical high altitude site. The focus of this paper is: (i) the quantification and characterization of key gross N turnover rates (i.e., mineralization, nitrification, microbial immobilization) and soil greenhouse gas (CO_2, N_2O, CH_4) exchange under different vegetation covers, and (ii) the influence of precipitation and freeze thaw cycles on biogeochemical processes.

Material and methods

Site characteristics and sampling design

Mount Kilimanjaro is located in Tanzania, next to the border of Kenya (2°45′ to 3°25′ S and 37°00′ to 37°43 E) and is the highest peak on the African continent (5895 m a.s.l.). Geologically it is a stratovolcano with a large spread of about 80 × 48 km (Downie et al. 1956). The study area (2500 m^2) represents a tropical alpine ecosystem (3°053,637′ S; 37°276,770′ E, 3880 m a.s.l.) is a slightly sloping area with no anthropogenic influence. The site is characterized by a diurnal climate with considerably high daily fluctuations in air temperature. The mean annual temperature is 5.3 °C and the mean annual precipitation is about 1417 mm (Appelhans et al. 2015a). The dominant vegetation species is alpine *Helichrysum* and a variety of mosses, herbs, and subalpine *Erica* shrubs (Hemp 2006) (Table 1). Thus, we defined three vegetation cover classes: low vegetation (low-veg),

Table 1 Classification (moss, herb, shrubs) and coverage of different plant species at non-vegetated, herb and shrub plots

Plot	Species	Mean cover class	Mean area cover	Vegetation type	Mean cover class	Mean area cover
Low veg	Mosses	+	< 5 %	Mosses	+	< 5 %
	Agrostis kilimandscharica	2	5–25 %	Herbs	1	5–25 %
	Haplosciadium abyssinium	+	< 5 %			
	Luzula abyssinica	2	5–25 %			
	Pentaschistis borussica	+	< 5 %			
	Pentaschistis minor	1	5–25 %			
	Alchemilla argylophylla	+	< 5 %	Shrubs	0	5–25 %
	Alchemilla johnstonii	0	< 5 %			
	Euryops dacrydiodes	+	< 5 %			
	Helichrysum citrispinum	+	< 5 %			
	Helichrysum forskhalii	r	< 5 %			
	Helichrysum newii	1	5–25 %			
	Helichrysum splendidum	1	< 5 %			
				Total	2	25–50 %
Herb	Mosses	+	< 5 %	Mosses	+	< 5 %
	Agrostis kilimandscharica	1	5–25 %	Herbs	2	25–50 %
	Haplosciadium abyssinium	+	< 5 %			
	Luzula abyssinica	1	5–25 %			
	Pentaschistis minor	+	5–25 %			
	Alchemilla argyrophylla	1	5–25 %	Shrubs	3	50–75 %
	Alchemilla johnstonii	+	< 5 %			
	Alchemilla microbetula	+	< 5 %			
	Erica trimera	r	< 5 %			
	Euryops dacrydiodes	1	5–25 %			
	Helichrysum citrispinum	1	5–25 %			
	Helichrysum forskhalii	2	5–25 %			
	Helichrysum newii	1	5–25 %			
	Helichrysum splendidum	r	< 5 %			
				Total	4	50–75 %
Shrub	Mosses	1	5–25 %	Mosses	1	5–25 %
	Agrostis kilimandscharica	+	5–25 %	Herbs	+	< 5 %
	Haplosciadium abyssinium	+	< 5 %			
	Luzula abyssinica	+	< 5 %			
	Alchemilla argyrophylla	r	< 5 %	Shrubs	4	> 75 %
	Alchemilla johnstonii	+	< 5 %			
	Erica trimera	4	50–75 %			
	Helichrysum citrispinum	+	< 5 %			
	Helichrysum newii	1	5–25 %			
				Total	4	> 75 %
1) r	< 5 %	Single individual of the species with less than 5 % coverage				
2) +	< 5 %	2–20 individuals of a species and collectively cover less than 5 %				
3) 1	< 5 %	Numerous individuals of a species collectively cover less than 5 %				
4) 2	5 % – 25 %	Species cover 5 % and 25 %				
5) 3	25 % – 50 %	Species cover 25 % and 50 %				
6) 4	50 % – 75 %	Species cover 50 % and 75 %				
7) 5	75 % – 100 %	Species cover 75 % and 100 %				

Coverage is expressed as a percentage contribution (*area coverage*) and classified (*cover class*) in the Braun-Blanquet scale, adapted by Mueller-Dombois and Ellenberg (1974)

herbal vegetation (herb) and shrub vegetation (shrub) (Fig. 1). Regarding these categories, areal coverage was calculated from Google Maps satellite images by unsupervised k-means clustering, resulting in 40.5 % low-veg (10 cm height), 51.9 % herbs (30 cm height) and 7.6 % shrubs (260 cm height) (Table 2) over a total site area of 50 × 50 m (Appelhans and Detsch 2015b). Within this area, three replicated plots per vegetation cover type (approximately 15 × 15 m; $N = 3 * 3 = 9$) were selected; each being represented by three randomly selected sampling locations (approximately 1.5 × 1.5 m; N = 3 * 9 = 27). At any of the nine plots replicated sampling locations were used to collect pooled samples for measurements of gross N turnover rates, GHG fluxes, microbial biomass, root abundance and other physicochemical soil properties (see section soil properties). At any of the 27 sampling locations the relative abundance of each plant species was recorded based on a visual estimation of the space a species covered in the 1.5 × 1.5 m area and expressed in the Braun-Blanquet scale, adapted by Mueller-Dombois and Ellenberg (1974). Information on the level of single plant species was aggregated and summarized as a relative abundance of shrubs, herbs and mosses as well as the total vegetation coverage for any of the three vegetation classes (Tables 1 and 2).

The soil is a Vitric Andosol (WRB 2014) characterized by partly shallow soil depths ranging from 5 to about 40 cm. Overall, an A-horizon of up to 10 cm depth was followed by either a B-horizon or bedrock (especially on surfaces without vegetation). An O-horizon was formed of the litter from the shrub vegetation.

Measurements of gross N turnover rates and GHG emissions were conducted between the 25th and 30th November 2014. As an additive treatment to the vegetation cover classes each of the 27 sampling locations was irrigated (2.5 mm m^{-2}) at the end of 27th November, in order to simulate the impacts of rainfall on N turnover processes and GHG emissions. Due to continuous heavy rainfall events soon after this irrigation event with even higher intensities during consecutive days, further irrigation was not necessary.

Gross N rates, dissolved inorganic N and organic C and N concentrations

For determination of gross N turnover rates, soil sampling and ^{15}N labeling of the soil was carried on the 25th (no rain) and the 28th (irrigation/rain) of November 2014. Gross N turnover rates were quantified using the ^{15}N pool dilution technique described by Rosenkranz et al. (2005) and (Davidson et al. 1992), with slight modifications. At any of the nine plots 300 g (composite of the three sampling locations) from the upper mineral soil (0–10 cm) were sampled. Bulk soil was sieved (5 mm mesh width, Dannenmann et al. 2006) and a subsample of 150 g was labeled either with 4.5 ml solution containing $(^{15}NH_4)_2SO_4$ or $K^{15}NO_3$ (50 atom% ^{15}N, N addition rate 3 mg N kg^{-1} dry soil) for investigation of gross N mineralization and nitrification rates, respectively. Isotope labeling of sieved soil was conducted by spraying the labeled solution on the soil as described by Dannenmann et al. (2009). One third of the ^{15}N labeled soil was extracted

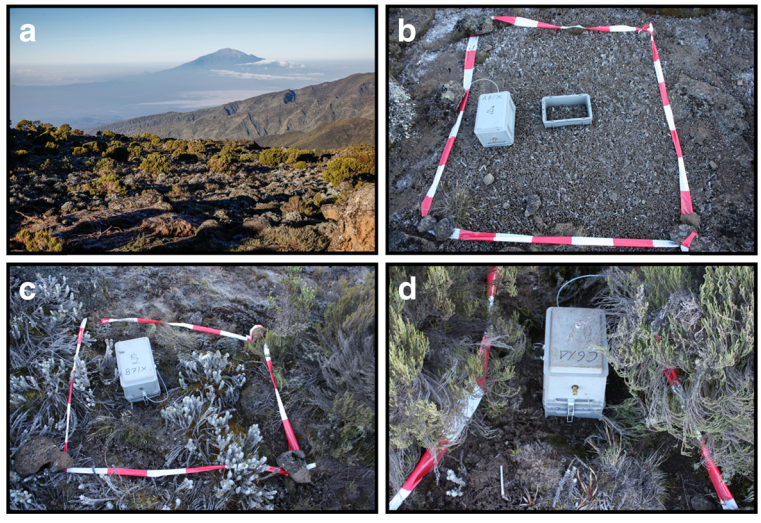

Fig. 1 Picture of the tropical alpine *Helichrysum* site (**a**) characterized by different vegetation classes (**b** = low-veg, **c** = herb and **d** = shrub)

Table 2 Top soil (0–10 cm) characteristics

Parameters		Low-veg	Herb	Shrub
NH_4^+-N	[μg N / g BTG]	1.25 ^{a} ± 0.25	2.72 ^{b} ± 0.35	1.19 ^{a} ± 0.11
NO_3^--N	[μg N / g BTG]	0.84 ^{a} ± 0.18	0.47 ^{ab} ± 0.18	0.20 ^{b} ± 0.13
DON-N	[μg N / g BTG]	23.46 ^{a} ± 1.14	26.66 ^{a} ± 2.24	30.79 ^{a} ± 5.63
Total extractable nitrogen	[μg N / g BTG]	25.55 ^{a} ± 1.37	29.85 ^{a} ± 2.57	32.03 ^{a} ± 5.53
Total extractable carbon	[μg C / g BTG]	429.03 ^{a} ± 63.2	390.31 ^{a} ± 79.12	314.79 ^{a} ± 35.84
SOC (0–10 cm)	[%]	6.16 ^{a} ± 0.94	10.87 ^{ab} ± 1.09	12.32 ^{b} ± 2.09
N (0–10 cm)	[%]	0.46 ^{a} ± 0.06	0.71 ^{a} ± 0.07	0.74 ^{a} ± 0.1
C:N ratio (0–10 cm)		12.86 ^{a} ± 0.44	15.00 ^{b} ± 0.23	16.13 ^{b} ± 0.61
MBN	[mg/kg]	25.76 ^{a} ± 4.43	61.26 ^{b} ± 6.25	69.77 ^{b} ± 14.29
MBC	[mg/kg]	367.79 ^{a} ± 32.79	606.43 ^{ab} ± 51.64	834.43 ^{b} ± 144.8
MBC:MBN ratio		16.86 ^{a} ± 2.09	10.13 ^{b} ± 0.32	12.98 ^{ab} ± 0.83
Bulk density	[g/cm3]	0.79 ^{a} ± 0.07	0.60 ^{b} ± 0.09	0.61 ^{b} ± 0.09
Stone content	[%]	11.17 ^{a} ± 2.4	1.47 ^{b} ± 0.81	2.33 ^{b} ± 1.09
pH		5.30 ^{a} ± 0.1	4.80 ^{b} ± 0.1	4.80 ^{b} ± 0.1
Live roots	[g l^{-1}]	0.75 ^{a} ± 0.14	0.51 ^{a} ± 0.1	0.92 ^{a} ± 0.19
Dead roots	[g l^{-1}]	0.07 ^{a} ± 0.02	0.36 ^{b} ± 0.04	0.25 ^{a} ± 0.11
Soil temperature (−2 cm)	[°C]	6,40 ^{a} ± 0,05	5,90 ^{b} ± 0,05	5,91 ^{b} ± 0,04
Soil temperature (−10 cm)	[°C]	6.21 ^{a} ± 0.02	7.08 ^{b} ± 0.02	5.83 ^{c} ± 0.01
VWC	[Vol.%]	30.17 ^{a} ± 2.56	27.56 ^{a} ± 2.60	26.37 ^{a} 0.93
Area coverage	[%]	40.50 ^{a}	51.90 ^{b}	7.60 ^{c}

DON dissolved organic nitrogen, *DOC* dissolved organic carbon, *TN* total extractable nitrogen, *TC* total extractable carbon, *SOC* soil organic carbon, *N* total soil nitrogen, *MBN* microbial nitrogen, *MBC* microbial carbon, *VWC* volumetric water content and area coverage of different vegetation classes of a tropical alpine *Helichrysum* site

Different superscript letters show significant differences between vegetation classes ($p \leq 0.05$)

15 min after labeling (t_1) and the second third incubated in-situ, covered with top soil layer material, for subsequent extraction 24 h (t_2) later (for details see Dannenmann et al. 2009). The remaining 50 g were used for the determination of volumetric soil water content (VWC) of the labeled soil. An additional 60 g of sieved unlabeled soil were used for measurements of VWC, dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) concentrations (Dannenmann et al. 2009). Further processing and analysis of soil extracts, such as: ^{15}N diffusion on acid traps, and analysis of isotopic signatures with an Elemental Analyzer coupled to an Isotope Ratio Mass Spectrometer (EA-IRMS) (Flash EA 1112 Series coupled to Finnigan Delta Plus XP, Thermo Fisher, USA); DIN (Epoch, BioTek Instruments Inc., USA) TN (total extractable nitrogen) and DOC (Multi N/C 3100, Analytik Jena, Germany) were carried out at laboratory facilities of KIT IMK-IFU (Garmisch-Partenkirchen, Germany) and followed the protocols described by Dannenmann et al. (2009). Gross N mineralization and nitrification rates and NH_4^+ and NO_3^- consumption were calculated using the equations given by Kirkham and Bartholomew (1954). Microbial immobilization of NH_4^+ was calculated as $^{15}NH_4^+$ consumption minus gross nitrification, assuming that gaseous losses and heterotrophic nitrification of organic N were negligible (Davidson et al. 1991. Microbial immobilization of NO_3^- was assumed to equal NO_3^- consumption. Based on the gained gross rates of inorganic N production and consumption, specific indicators of N cycling were calculated. The ratio of gross NH_4^+ immobilization plus gross NO_3^- consumption to gross N mineralization plus gross nitrification is referred to as relative N retention and the ratio of gross NH_4^+ immobilization to gross N mineralization is referred to as relative NH_4^+ immobilization.

GHG measurements

For GHG (CO_2, N_2O and CH_4) exchange measurements one static chamber (25.2 × 15.2 × 14.7 cm) was installed

at each of the 27 sampling locations. A rubber sealing and clamps maintained gas tightness of the chamber at collars driven 3 to 5 cm into the soil. The opaque polypropylene chambers were equipped with a rubber septum and a 30 cm long and 1/8 in. Teflon tubing to allow pressure equilibrations during sampling. Gas sampling was performed with a 60 ml gas tight syringe (Omnifix®, B. Braun, Melsungen, Germany) equipped with a one-way LuerLock stop-cock (VWR International, Darmstadt, Germany). Over the whole measuring campaign, four times a day (at 6:00, 9:00, 14:00 and 18:00) headspace gas was sampled at t1 = 0, t2 = 15, t3 = 30, t4 = 45 and t5 = 60 min after chamber closure in order to cover potential diurnal patterns. Sampling followed the gas pooling protocol of Arias-Navarro et al. (2013) by subsequently taking and mixing 15 ml gas samples from three replicated plot chambers at any sampling time (t1–t5) with one syringe. Thus, this approach integrates gas flux measurements at replicated sampling locations but still maintains plot replication. A total of 45 ml of pooled sample was used to flush and finally over-pressurize (5 ml) 10 ml glass vials (SRI Instruments, Bad Honnef, Germany). The samples were shipped to IMK-IFU (Garmisch-Partenkirchen, Germany) for further analysis using a headspace auto sampler (HT200H, HTA s.r.l, Brescia, Italy) coupled to a gas chromatograph (8610 C, SRI Instruments, Torrence, USA) equipped with an electron capture detector (ECD N_2O) and a flame ionization detector/methanizer (FID: CH_4 and CO_2). Samples were continuously calibrated with standard gas samples (N_2O: 406 ppb; CH_4: 4110 ppb; CO_2: 407.9 ppm, Air Liquide, Düsseldorf, Germany). Flux rates were calculated with R version 3.2.0 including HMR package 0.3.1 for calculation of GHG flux rates by linear increase or decrease in gas concentration over time (n = 5). Quality checks were applied and flux measurements were discarded at $r^2 < 0.6$. Mean detection limits (MDL) calculated according to Parkin et al. (2003) were 0.17 mg CO_2-C, 5.3 μg, CH_4-C or 0.6 μg N_2O -N m^{-2} h^{-1}, respectively.

Microbial biomass and fine root biomass

Soil samples were taken from 27 sampling locations (nine per vegetation class) with a steel corer (5 cm diameter) to a depth of 10 cm and separated into two depths: 0–5 cm and 5–10 cm. In three low-veg plots we could only take samples to 5 cm and 2.5 cm depth because of underlying bedrock material. Samples were transferred into plastic bags and transported to the laboratory in Nkweseko station, Tanzania, and stored at 5 °C. Processing of the samples was done within 60 days. All the macroscopically visible roots longer than 10 mm were extracted by hand with tweezers. The method described by Van Praag et al. (1988) and modified by Hertel and Leuschner (2002) was inapplicable under field conditions. Thus, roots were separated to those belonging to shrubs and the ones from grasses, herbs and mosses under the stereomicroscope. Also, we distinguished between live roots (biomass) and dead roots (necromass) by root elasticity and degree of cohesion of cortex, periderm and stele. An indicator of root death is a non-turgid cortex and stele, or only the presence of the periderm (Leuschner et al. 2001). Fine root biomass and necromass samples were dried at 70 °C (48 h) and weighed. After the separation of roots, soil samples were stored in 60 ml PE-Tubes (VWR, Germany) at 4 °C and shipped to Göttingen (Germany) for further analysis. Microbial biomass C (MBC) and microbial biomass N (MBN) were quantified by fumigation-extraction method following the protocol introduced by Vance et al. (1987).

Measurement of soil properties

All physicochemical soil properties were measured from pooled samples (N = 3) at any of the three replicated vegetation plots (N = 9). Soil pH was measured from air-dried soil samples dissolved in 0.01 M $CaCl_2$ solutions with a SenTix 61 electronic pH-meter (WTW GmbH, Weilheim, Germany). Bulk density (BD) was calculated from oven dried (72 h at 105 °C) undisturbed soil cores (100 cm^3) taken at 0 to 5 cm soil depth. From the same samples the stone fraction was measured as water displacement of stones >2 mm. The C and N contents were determined using an automated C:N analyzer (Vario EL cube, Elementar, Germany). About 40 mg of dry soil were fine ground and combusted at 950 °C and the evolved CO_2 and NO_x were measured using a thermal conductivity detector.

Soil temperature was continuously (1 min intervals) measured at 2 and 10 cm soil depth over the whole measuring campaign at 27 sampling locations (EBI 20-TH1; ebro Eletcronic, Ingolstadt, Germany). Means were calculated per vegetation class and soil depth. In addition to the determination of VWC from soil samples used for quantification of N turnover rates, VWC was also measured after GHG measurements in any

chamber by a portable frequency domain sensor (GS3, Decagon Devices©, Pullman, USA).

Statistics

Kolmogorov–Smirnov statistics were applied to test for normal data distribution for any measured parameter. Since neither N gross turnover rates nor GHG emissions were normally distributed, we applied log transformation on N gross turnover rates and square root transformation on GHG data. Differences between the no-rain and irrigation/rainfall treatments for all sites were assessed using independent-samples t-test. For GHG data a two-way analysis of variance (ANOVA) (Tukey's HSD) was conducted to test differences in time and between vegetation classes. Additionally, a one-way ANOVA (Tukey's HSD) was executed for N turnover rates and all other soil parameters to test for differences between vegetation classes. Correlation analyses between GHG, N turnover and soil parameters were conducted across all nine plots using Pearson product-moment correlation coefficient. For identification of the main controls on N gross rates and GHG emissions multiple stepwise regression analysis was applied. Level of significance was chosen at $p < 0.05$. All statistical analyses were calculated with IBM® SPSS® statistics 21 (IBM Corporation, New York, USA).

Results

Soil properties

The temperatures at 2 cm soil depth showed a strong diurnal cycle with a maximum of up to 22 °C around noon and minimum of 0 °C in the early morning hours. Even though the soil surface was covered with frost, minimum temperatures in 2 cm soil depth were slightly higher than 0 °C. Overall in 2 cm soil depth the mean diurnal temperature variation of 15 °C was much higher compared to the temperature differences between the vegetation classes which were mostly <1 °C. The temperature at 10 cm soil depth showed a dampened diurnal variation with temporarily delayed maximum (12 °C) and minimum temperatures (3 °C) and a more pronounced difference (2 °C) across the three vegetation classes (Fig. 2). Over the whole measuring campaign mean soil temperatures at 2 and 10 cm soil depth ranged between 5.9 and 7.1 °C with significantly higher values found at 2 cm depth at the low-veg sites and at 10 cm depth at the herb plots (Table 2).

In contrast to soil temperature, the temporal variation of VWC at all three vegetation classes was minor, even though soils were exposed to one irrigation and consecutive rainfall events after the 28th November 2014 (Fig. 2). For the low-veg and herb plots mean daily VWC ranged between 22 and 40 vol% with a tendency of decreasing VWC from beginning of the measuring campaign. VWC

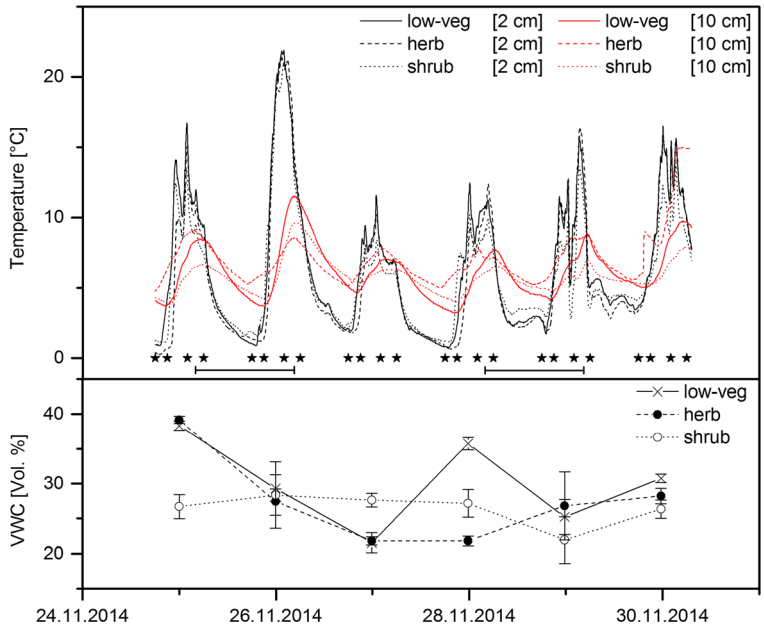

Fig. 2 Course of soil temperature (at 2 and 10 cm) and VWC (0 to 5 cm) at three vegetation classes of the tropical alpine *Helichrysum* site. Stars represent gas sampling times and lines below the stars the incubation time for the ^{15}N labeled soil

at the shrub plots did not vary significantly over time and ranged between 26 and 28 vol%. Only the low-veg treatment showed an increase of VWC after irrigation. Mean VWC of the low-veg, herb and shrub treatments, measured daily at the GHG chamber positions, were not significantly different (Table 2) and were in the same range as VWC measurements calculated from soil samples used for quantification of gross N turnover rates (Fig. 3).

Measurement of pH revealed more acidic conditions for the herb and shrub plots than for low-veg plots. The BD was higher for the low-veg plots (0.8 g cm^{-3}) compared to the herb and shrub plots (0.6 g cm^{-3}), whereas the C and N content as well as C/N ratio increased with vegetation cover (Table 2).

Gross N turnover rates and extractable soil C and N concentrations

At the first sampling time under no rain conditions gross N mineralization significantly increased with vegetation cover (Fig. 3a). Rates on the herb plots were four-times higher and on shrub plots more than five-times higher than on the low-veg plots. Gross nitrification rates showed the same, though not significant trend as N mineralization rates but were four-times lower than gross N mineralization rates on the low-veg and about ten-times lower than on the vegetated plots. NH_4^+ immobilization rates significantly increased with growing vegetation cover. Gross NO_3^- consumption rates showed the same trend but were found to be much lower than NH_4^+ immobilization rates (Fig. 3b).

For the sampling after the irrigation/rain event, the magnitude and trends of gross N mineralization and nitrification rates across the three treatments were comparable to the no-rain situation. However, plant effects were less pronounced, which resulted in diminished statistical significance of the differences across the vegetation cover treatments (Fig. 3e). The same was true for NH_4^+ immobilization rates, which were slightly lower in the vegetated plots when compared to the no-rain situation. NO_3^--consumption rates declined after irrigation/rainfall and were detectable only in the shrub treatment.

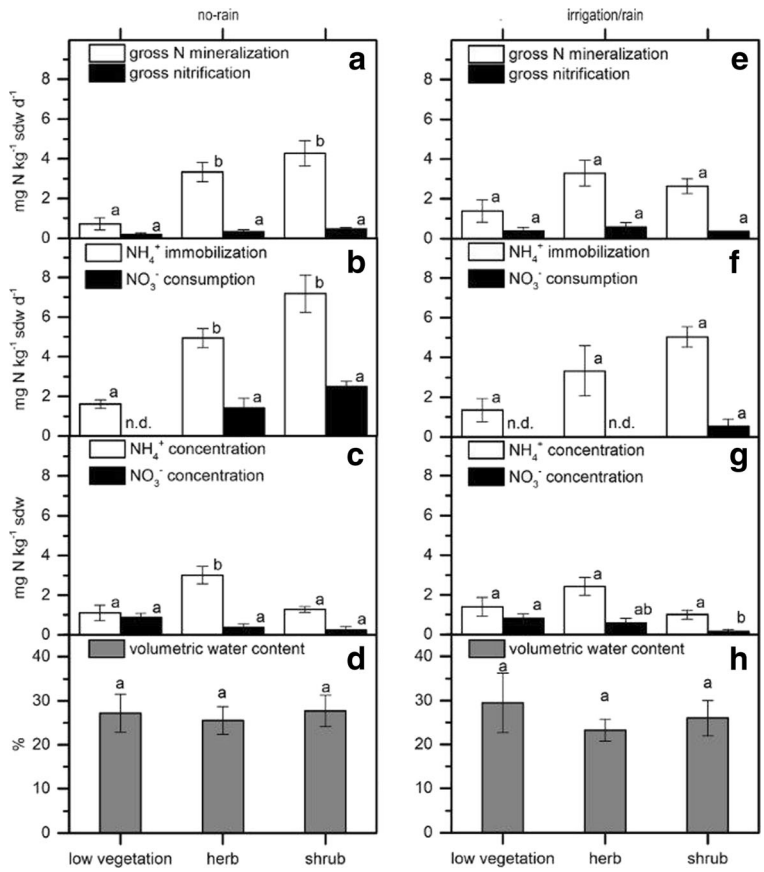

Fig. 3 Gross N-turnover rates, soil N concentration and water content at three vegetation classes of the tropical alpine *Helichrysum* site. **a**–**d** represent measurements for no-rain, **e**–**h** represent measurements after irrigation (rain). Stars indicate times of GHG chamber measurements; lines indicate incubation time of gross N turnover measurements. Error bars represent standard errors of the mean. Lower case letters represent significant differences ($p < 0.05$) between the vegetation classes

Before irrigation NH_4^+ and NO_3^- concentrations (Fig. 3c) showed a different pattern across the three treatments than gross N turnover rates. NH_4^+concentrations were highest at the herb plots, while NO_3^- concentrations even showed a decreasing trend with increasing vegetation cover. After irrigation/rainfall mineral N concentrations were slightly lower but showed the same trends compared to the no-rain sampling (Fig. 3g). Across all vegetation classes NO_3^- concentrations were persistently lower than NH_4^+ concentrations, irrespective of irrigation/rainfall (Fig. 3c and g). Overall, DON concentrations were more than ten-times higher than DIN concentrations at the *Helichrysum* site. DON concentrations did not differ significantly between treatments but nevertheless showed an increasing trend with increasing vegetation cover (Table 2).

Both relative N retention (Table 3) as well as relative NH_4^+ immobilization significantly increased in the presence of shrub vegetation compared with the low-veg plots in the irrigation/rain treatment, but were not significantly affected by vegetation in the no-rain treatment (Table 3).

Soil GHG emissions CO_2, CH_4 and N_2O emissions

Since soil GHG emissions did not show any significant changes to the irrigation/rainfall event, data were aggregated over the whole measuring campaign (Table 4), and for evaluation of diurnal patterns were divided into four classes representing different hours of the day (Fig. 4).

Soil CO_2 emissions were low and ranged between 3.3 and 28.3 mg C m^{-2} h^{-1}. Emissions were significantly higher on the herb and shrub plots compared to the low-veg plots (Table 4). At all plots, the highest CO_2 fluxes were measured at 2 pm and the lowest fluxes occurred at 6 am. This diurnal pattern was most obvious for the herb plots, which also showed highest daily maximum fluxes (Fig. 4a). The difference between minimum and maximum fluxes at the shrub plots was lower than the herbs plots but still higher than at the low-veg plots, which showed only a minor diurnal pattern. For all three vegetation classes chamber measurements revealed a net uptake of CH_4 into the soil, with rates ranging between −4.9 and −45.7 μg CH_4-C m^{-2} h^{-1} (Table 4). At the herb and shrub plots, uptake rates were significantly higher (approximately 50 %) than at the low-veg plots (Table 4). At medium and highly vegetated plots diurnal patterns of fluxes were less pronounced than for CO_2 emissions and were non-existent at low-veg plots (Fig. 4b). For all vegetation classes N_2O emissions were below the detection limit (0.6 μg N_2O-N m^{-2} h^{-1}) and showed no diurnal pattern (Fig. 4c, Table 4).

Microbial biomass (N and C) and fine root biomass

Microbial biomass N was significantly lower at low-veg plots compared to herb and shrub plots (Table 2). Microbial biomass C showed a comparable pattern across vegetation treatments; however, the only significant differences existed between the low-veg and shrub plots. Overall, at all vegetation classes, biomass of live roots was much higher than biomass of dead roots. Dead root abundance was significantly higher at the herb plots than at the low-veg and shrub plots. In contrast, the abundance

Table 3 N turnover indicators for the three vegetation classes for no-rain, irrigation/rain and combined conditions

	Vegetation class	$Nret_{rel}$	$ImmNH_4^+{}_{rel}$
No rain	Low-veg	2.59 ^{aA} ± 0.85	3.45 ^{aA} ± 1.12
	Herb	1.74 ^{aA} ± 0.15	1.53 ^{aA} ± 0.16
	Shrub	2.07 ^{aA} ± 0.08	1.69 ^{aA} ± 0.06
Irrigation/ rain	Low-veg	0.55 ^{aB} ± 0.41	0.96 ^{aA} ± 0.22
	Herb	0.70 ^{abB} ± 0.22	0.92 ^{aB} ± 0.18
	Shrub	1.89 ^{bA} ± 0.2	1.93 ^{bA} ± 0.09
Combined	Low-veg	1.26 ^{a} ± 0.75	2.21 ^{a} ± 0.55
	Herb	1.22 ^{a} ± 0.17	1.23 ^{a} ± 0.07
	Shrub	1.74 ^{a} ± 0.09	1.82 ^{a} ± 0.08

Superscript lower case letters represent significant differences ($p < 0.05$) between vegetation classes

Superscript capital letters represent significant differences ($p < 0.05$) of no-rain and irrigation/rain within one vegetation class

$Nret_{rel}$ relative N retention, $ImmNH_4^+{}_{rel}$ relative NH_4^+ immobilization

Table 4 Compilation of minimum, mean, maximum and area weighted mean fluxes of CO_2 (mg C m^{-2} h^{-1}), CH_4 (μg C m^{-2} h^{-1}) and N_2O (μg N m^{-2} h^{-1}) for different vegetation classes and the whole *Helichrysum* ecosystem

GHG emission	Vegetation class	Min.	Max.	Mean
CO_2 [mg C m^{-2} h^{-1}]	Low-veg	3.38	14.60	7.20 ^{a} ± 0.55
	Herb	3.85	28.32	11.54 ^{b} ± 0.71
	Shrub	4.96	17.42	10.86 ^{b} ± 0.56
	Area weighted total			9.73 ± 0.63
CH_4 [μg C m^{-2} h^{-1}]	Low-veg	−3.64	−33.14	−15.37 ^{a} ± 2.24
	Herb	−4.91	−45.71	−22.44 ^{ab} ± 1.70
	Shrub	−9.04	−33.90	−23.75 ^{b} ± 1.78
	Area weighted total			−19.68 ± 1.92
N_2O [μg N m^{-2} h^{-1}]	Low-veg	−2.69	3.48	0.25 ^{a} ± 0.23
	Herb	−1.48	1.65	0.20 ^{a} ± 0.13
	Shrub	−0.83	4.01	0.11 ^{a} ± 0.16
	Area weighted total			0.21 ± 0.17

Superscript letters show significant differences between vegetation classes ($p \leq 0.05$)

of live roots did not differ across vegetation treatments and herb plots tended to have lowest values (Table 2).

Correlation and controls of gross N turnover rates and GHG emissions

Both N mineralization and nitrification were positively correlated with soil CO_2 emissions, but surprisingly no correlation was found between them. In addition, N mineralization was also positively correlated with NH_4^+ immobilization and NO_3^- consumption. Also, for the latter two, a high positive correlation was found (Table 5). Stepwise linear regression revealed total extractable N, soil NO_3^-/ NH_4^+ concentration and MBN as the main parameters controlling gross N turnover rates. The highest r^2 (> 0.9) of the regression was found for N mineralization and NH_4^+ immobilization by combination of three of the before mentioned parameters (Table 6). NO_3^- consumption as well as indicators of N cycling could be best explained either by soil NO_3^- or NH_4^+ concentrations, although with much lower predictive power ($r^2 < 0.5$). Note that nitrification, N_2O and CH_4 emissions could not be explained by any of the parameters.

Discussion

In the tropical alpine Helichrysum ecosystem variations in air and soil temperature are rather driven by diurnal (diff. 20 °C) than seasonal patterns (diff 2 °C of warmest and coldest month). Even though rainfall has a more pronounced seasonal pattern than air temperature, changes in soil moisture are expected to be low as was demonstrated by insignificant differences between the no-rain and irrigation/rain treatment (Table 7). Overall, this is related to a high vertical water percolation caused by high porosity and cleaved bedrock material. Due to this specific climate and soil conditions, we are convinced that the short term character of our study is not limiting the representativeness of our main findings also for longer time scales. In contrast to soil temperature and moisture, vegetation cover exerted pronounced effects on gross N turnover rates and GHG emissions. Accordingly, the following discussion focuses mainly on effects of vegetation cover.

Gross N turnover rates

Our approach of quantifying gross rates of N turnover together with extractable organic and mineral C and N substrates allowed a previously unavailable functional insight into N cycling in the *Helichrysum* ecosystems at Mt. Kilimanjaro. Overall, the N cycle was characterized by more than an order of magnitude larger DON than mineral N availability, by high NH_4^+ immobilization rates and small nitrification rates with minimal soil NO_3^- concentrations, accompanied by an overall high microbial inorganic N retention capacity. This characterizes a rather undisturbed, N-limited and thus closed N cycle, which is confirmed also by extremely low N_2O

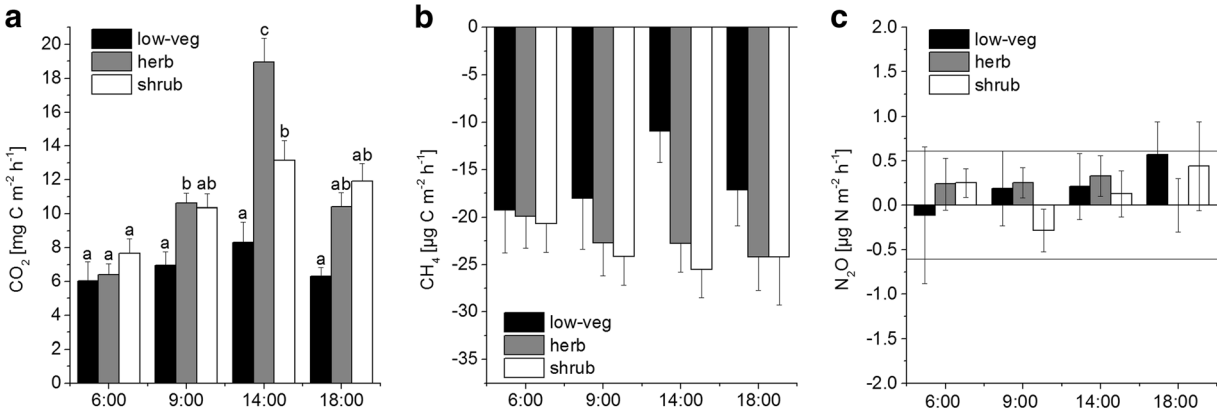

Fig. 4 Diurnal patterns of soil GHG exchange (A = CO_2, B = N_2O, C = CH_4) at three vegetation classes of the tropical alpine *Helichrysum* site. Error bars represent standard error of the mean. Letters indicate significant ($p < 0.05$) temporal differences of fluxes within a vegetation class. Note: no letters are presented for CH_4 and N_2O since no significant differences were detected. Lines at 0.6 and −0.6 in Fig. 4c, represent the MDL for N_2O measurements. Correlation coefficients of soil CO_2 emissions and temperature were 0.53 ($p < 0.01$), 0.88 ($p < 0.001$), 0.67 ($p < 0.001$) for low-veg, herb and shrub plots, respectively

emissions. Nevertheless, the high DON versus low mineral N availability is challenging the current paradigm of the N cycle: that depolymerization of organic macromolecules is the dominant "bottleneck" of overall N cycling (Schimel and Bennett 2004). At least for the tropical alpine *Helichrysum* ecosystem under investigation, nitrification seems to be the limiting step of overall N cycling.

Gross N mineralization rates (Table 7) were considerably higher on the vegetated plots and agree well with data compiled by Booth et al. (2005) for arctic/montane grassland ecosystems and Cookson et al. (2002) for winter conditions of soils in temperate regions. The area weighted gross nitrification rate for the *Helichrysum* site (Table 7), including all vegetation classes, is much lower but in the same range as rates reported for an N-limited beech forest soil in southern Germany (Dannenmann et al. 2006). However, the latter as well as other studies that report boreal and alpine ecosystem nitrogen turnover processes (Clein and Schimel 1995; Jaeger et al. 1999; Kielland et al. 2006; Schütt et al. 2014), are hardly comparable to the *Helichrysum* ecosystem. This is mainly due to different climatic (e.g., temperature, precipitation, and radiation regimes) and vegetation characteristics (i.e., larger vegetation cover, higher litter input and decomposition rates) as compared to the *Helichrysum* site. Similarly, vegetation dependent variation of soil properties can also be observed at the site scale in our study (i.e., between the vegetation cover types at our *Helichrysum* site). Since larger vegetation cover leads to an increase in litter production and dead roots in the soil, SOM contents were found to increase with vegetation cover (Table 2), a finding in line with other studies (e.g., Prescott 2010). Such plant-soil interactions provide an explanation for the observation of increased microbial biomass and gross N turnover rates with higher SOC contents (e.g., Geßler et al. 2005; Pabst et al. 2013), as was observed in our *Helichrysum* ecosystem (Table 7). Results of the regression analysis support this finding. From the total set of soil environmental parameters (except nitrogen substrate) only MBN and SOC were selected as the main controls for the dominating N processes of N mineralization and NH_4^+ immobilization.

The very low relative importance of nitrification versus NH_4^+ immobilization facilitated the overall closed N cycle in the *Helichrysum* ecosystem. Though it is reported that nitrification might be more sensitive to low temperatures than ammonification (Cookson et al. (2002), the low nitrification rates identified in this study may also be related to the high DOC availability, which favors heterotrophic microbial NH_4^+ immobilization over gross autotrophic nitrification (Butterbach-Bahl and Dannenmann 2012). The trend of declining DOC with growing vegetation cover might also be explained by heterotrophic microbial NH_4^+ immobilization, which is, in contrast to the mainly autotrophic nitrification, a carbon consuming process (Rennenberg et al. 2001). The positive correlation between CO_2 fluxes and N mineralization and the lack of correlation between nitrification and N mineralization (Tables 5 and 6) contradicts the general findings of other studies (summarized

Table 5 Pearson's correlation coefficients (R) between N gross turnover rates and CO_2 emissions

	N mineralization	Nitrification	NH_4^+ immob.	NO_3^- cons.
CO_2	0.76*	0.74*	0.59	0.42
N mineralization		0.25	0.94**	0.75**
Nitrification			0.16	0.29
NH_4^+ immob				0.88**

NH_4^+ immob immobilization and NO_3^- cons consumption

*p < 0.05, **$p < 0.01$

by Booth et al. 2005). However, it supports the assumption of dominant heterotrophic microorganisms versus autotrophic nitrifiers. Heterotrophic microorganisms use NH_4^+ solely for growth, whereas autotrophic nitrifiers need NH_4^+ also for energy production, impairing their competition for NH_4^+ against microbial NH_4^+ immobilization at high DOC over N availability (Verhagen and Laanbroek 1991; Booth et al. 2005; Dannenmann 2007). This suggests that increased N turnover rates at vegetated plots, caused by higher litter production and rhizodeposition (Hodge et al. 2000; Schimel and Bennett 2004; Phillips et al. 2011; Kuzyakov and Blagodatskaya 2015), do not enhance the risk of N loss as long as the C:N ratio is not narrowing. In contrast, plants may even further compete with nitrification for soil NH_4^+. In this context, increasing microbial inorganic N immobilization (Table 7) and N retention capacity (Table 3) at shrub plots implies an intense plant-microbe competition for the limited N resources. This is further confirmed by declining NO_3^- concentrations and residence time of NH_4^+ (i.e., the ratio of NH_4^+ concentration to ammonification) with increasing vegetation cover

Table 6 Multiple regression analysis for identification of main environmental controls on gross N processes and greenhouse gas emissions

	Parameter	Coefficient	Change in R^2	*p* value	Multiple R^2	Adjusted R^2	p value
Gross N mineralization	Intercept	−17.858			0.947	0.928	<0.001
	TN	13.694	0.605	<0.001			
	NO_3^-	−0.697	0.896	0.018			
	MBN	0.045	0.947	0.004			
Gross nitrification	Nossne						
NH_4^+ immobilization	Intercept	−16.431			0.951	0.93	<0.001
	NO_3^-	−2.824	0.544	<0.001			
	TN	11.849	0.872	0.001			
	SOC	0.119	0.951	0.12			
NO_3^- consumption	Intercept	−0.418			0.804	0.782	<0.001
	NO_3^-	−1.498	0.804	<0.001			
Rel. N retention	Intercept	0.028			0.402	0.335	0.036
	NO_3^-	−0.177	0.036	0.036			
Rel. NH_4^+ immob.	Intercept	2.616			0.479	0.422	0.018
	NH_4^+	0.512	0.479	0.018			
CO_2 flux	Intercept	5.901			0.46	0.382	0.045
	MBN	0.055	0.682	0.045			
N_2O flux	None						
CH_4 flux	None						

Discarded parameters ($p > 0.05$) were NH_4^+, NO_3^-, DON, total extractable N, total extractable C, SOC, N, MBC, live roots, and dead roots

TN total extractable nitrogen, *NO_3^-* soil NO_3^- concentration, *NH_4^+* soil NH_4^+ concentration, *SOC* soil organic carbon, *MBN* microbial biomass N

Table 7 Mean (no-rain and irrigation/rain treatment) gross N-turnover rates for three vegetation classes and for the whole (area weighted mean) *Helichrysum* ecosystem

	Low-veg	Herb	Shrub	Area weighted mean
	[μg N g^{-1} SDW d^{-1}]			
Gross N mineralization	1.05 ^{a} ± 0.3	3.31 ^{b} ± 0.35	3.58 ^{b} ± 0.46	2.42 ± 0.8
Gross nitrification	0.29 ^{a} ± 0.09	0.46 ^{a} ± 0.11	0.42 ^{a} ± 0.04	0.39 ± 0.05
NH_4^+ immobilization	1.48 ^{a} ± 0.27	4.13 ^{b} ± 0.65	6.26 ^{c} ± 0.64	3.22 ± 1.38
NO_3^- consumption	n.d. n.d.	0.49 ^{ab} ± 0.44	1.65 ^{b} ± 0.41	0.38 ± 0.58
	[kg N ha^{-1} d^{-1}]			
Gross N mineralization	0.83 ^{a} ± 0.29	1.97 ^{b} ± 0.7	2.17 ^{b} ± 0.82	1.52 ± 0.42
Gross nitrification	0.23 ^{a} ± 0.08	0.27 ^{a} ± 0.1	0.26 ^{a} ± 0.09	0.25 ± 0.01
NH_4^+ immobilization	1.17 ^{a} ± 0.41	2.46 ^{b} ± 0.87	3.80 ^{c} ± 1.44	2.04 ± 0.76
NO_3^- consumption	n.d. n.d.	0.29 ^{ab} ± 0.1	1.00 ^{b} ± 0.38	0.23 ± 0.37

Superscript lower case letters represent a significant difference ($p < 0.05$) between vegetation classes

(Fig. 3). Even though intense microbial competition may reduce short-term plant N availability, the process of internal N recycling along microbial loops also enables ecosystem nitrogen retention. This can even lead to sustainable nitrogen provision to plants, since plants on the long-term may compete better versus microbes due to their longer and higher N storage capacity (Kuzyakov and Xu 2013, Hodge et al. 2000).

Currently, about 60 % of the *Helichrysum* system is covered with vegetation. Palaeosols reflecting movements of the vegetation belts caused by palaeoclimatic fluctuations (Zech 2006; Zech et al. 2014) show that climate change may induce an increase in vegetation cover in the *Helichrysum* ecosystem. Since N turnover rates are highest at vegetated plots (Table 7), this may increase gross N turnover rates; however, based on our findings this does not necessarily open the N cycle. Therefore, the *Helichrysum* ecosystem may be rather vulnerable to expected increases of atmospheric N deposition in tropical regions of Africa (Dentener et al. 2006; Vitousek et al. 1997), which may narrow the soil C:N ratio and thus could increase nitrification, transiently opening the N cycle of the previously undisturbed ecosystem.

GHG emissions

The area weighted mean CO_2 flux measured for the *Helichrysum* ecosystem was 86.4 g CO_2-C m^{-2} $yr.^{-1}$, which is only slightly higher than soil respiration rates reported for Tundra ecosystems (60 g CO_2-C m^{-2} $yr.^{-1}$; Raich and Schlesinger 1992). As decreasing temperatures inhibit soil respiration, we assume that similar to Tundra ecosystems, soil respiration of the *Helichrysum* ecosystem at Mt. Kilimanjaro is mainly temperature limited. The total CO_2 production in intact soils is the sum of respiration from soil organisms, roots and mycorrhizae. Litter production, dead root decomposition and root exudates increase the organic matter inputs and thus soil respiration rates (Raich and Schlesinger 1992). Significant differences in organic matter inputs reflected by higher SOC contents at herb and shrub plots and the highest live root abundance at shrub plots explain the increase of soil CO_2 emissions with increasing vegetation cover. Root respiration is positively correlated to temperature (Luo and Xuhui 2006) and solar radiation, the latter triggering root respiration via photosynthesis and subsequent stimulation of root exudation (Kuzyakov and Gavrichkova 2010). This is supported by our findings by more pronounced diurnal patterns of soil CO_2 emissions at the vegetated plots (Fig. 4a). The slightly lower emissions from the shrub plots might be caused by lower soil temperatures during the daytime due to greater shading compared to herb plots (Figs. 1 and 2). The minor influence of root respiration and lower SOM content leads to the lowest temperature sensitivity of CO_2 emissions at the low-veg plots, which is also represented in the lower correlation coefficient with soil temperature (Fig. 4a). Aside from soil temperature, soil moisture was also found to correlate positively with soil respiration (e.g., Davidson et al. 1998; Raich and Tufekciogul 2000). Due to the high percolation rates, changes in soil moisture caused by irrigation/rainfall events were dampened, and neither impacted N turnover rates nor GHG emissions. From this one can

conclude that soil N and C cycling in the tropical alpine *Helichrysum* ecosystem is mainly controlled by changes in soil temperature.

During the whole measuring campaign, all vegetation classes within the *Helichrysum* ecosystem were a net-sink for atmospheric CH_4. The area weighted mean uptake rate of 1.72 kg C ha^{-1} $yr.^{-1}$ is higher than the mean uptake rate of 1.12 kg C ha^{-1} $yr.^{-1}$ reported for Tundra ecosystems (Dutaur and Verchot 2007), indicating a high adaptation of microorganism to the specific climatic and soil conditions. CH_4 uptake in soils is driven by oxidation via methanotrophic microorganisms (Conrad 1996; Butterbach-Bahl and Papen 2002), which is primarily influenced by diffusive properties regulating the availability of atmospheric CH_4 and oxygen in the soil (Ball et al. 1997; Boeckx et al. 1997) and therefore occurs predominantly in the top-soil (Bender and Conrad 1994; Steinkamp et al. 2001). The significantly lower CH_4 uptake rates on the low-veg plots may result from generally lower soil aeration caused by significantly higher soil BD (Table 2). In addition, during the observation period, soil moisture was highest at the low-veg plots (Fig. 2), which further reduced gas exchange with the atmosphere and thus lowered O_2 and CH_4 supply for methanotrophic microorganisms. Due to favorable physical soil conditions, observed CH_4 uptake rates are highest in forest ecosystems (Dutaur and Verchot 2007; Adamsen and King 1993; Castro et al. 1995), which is further supported by Matzner and Borken (2008), who pointed out that vegetation generally enhances soil diffusivity. Various studies also showed a positive correlation between temperature and CH_4 uptake rates, in particular for forest ecosystems (Butterbach-Bahl and Papen 2002; Kiese et al. 2008). Likewise, CH_4 fluxes at the vegetated plots show a weak diurnal trend with lowest uptake rates generally occurring at 6 am (Fig. 4b). Contradictory to our hypothesis there was no impact of irrigation/rainfall on CH_4 uptake at any of the three vegetation classes, which again can be attributed to the shallow soils and the high water drainage capacity.

The majority of N_2O fluxes in the *Helichrysum* ecosystem are below the mean detection limit, showing that N_2O emissions are negligible in the *Helichysum* ecosystem. N_2O production end emissions in soils predominantly occur indirectly via nitrification and directly via denitrification (Conrad 1996; Butterbach-Bahl et al. 2013). Since in our study nitrification rates are very low and denitrification proceeds mainly under anaerobic soil conditions at WFPS >70 % (Butterbach-Bahl et al. 2013; Silver et al. 2001), none of these two relevant processes could produce significant amounts of N_2O. Contrary to our hypothesis, neither the vegetation nor irrigation/rainfall affected the magnitude of N_2O emissions. N_2O emissions were assumed to be higher on the vegetated plots since former studies revealed higher microbial biomass and activity as well as increased N-turnover to be positively correlated with N_2O emissions (e.g., Butterbach-Bahl et al. 2011). Due to the high rates of microbial NH_4^+ immobilization and high relative N retention (indicating low nitrogen availability particularly at vegetated plots (Tables 3 and 7), the increase of N_2O emissions with vegetation cover was likely hampered at the investigated *Helichrysum* ecosystem.

Contrary to our assumption, daily freeze-thawing was existent only in the soil surface and, thus in combination with low N availability did not affect the magnitude of N_2O emissions as reported for other ecosystems under similar climatic conditions (e.g., Holst et al. 2008). Since N_2O fluxes did not increase with vegetation cover, progressive warming and the potentially associated expansion of vegetation will have only minor impacts on the overall N_2O budget of the *Helichrysum* ecosystem.

Conclusions

Our study is the first presenting N turnover processes and GHG exchange in an afro-alpine tropical ecosystem. N turnover at the investigated *Helichrysum* ecosystem is primarily temperature controlled and less affected by changes in soil moisture due to shallow, well-draining soils. SOM input from the vegetation and root exudates increases C and N substrate availability, thus increasing microbial biomass and activity in vegetated patches. Overall this leads to higher N mineralization rates favoring subsequent microbial NH_4^+ immobilization. The high N retention and the low DIN concentrations reveal strong microbial competition for N, and thus, potential N limitation for plant growth. This indicates a rather closed N cycle, which is confirmed by the extremely low N_2O emissions. Most striking are the low nitrification rates, which seem to limit overall N cycling in the *Helichrysum* ecosystem. Nitrogen cycling will be accelerated if vegetation cover expands with progressive warming. Since this does not necessarily open the N cycle, the *Helichrysum* ecosystem may be rather vulnerable to the expected increase in atmospheric N deposition. The latter could lead to a narrowing of the soil C:N

ratio and, thus, may increase nitrification and transiently open the N cycle, which means losses of N to the atmosphere and waters of the previously undisturbed *Helichrysum* ecosystem.

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References

Adamsen AP, King GM (1993) Methane consumption in temperate and subarctic forest soils: rates, vertical zonation, and responses to water and nitrogen. Appl Environ Microbiol 59:485–490

Alm J, Saarino S, Nykänen H, Silvola J, Martikainen PJ (1999) Winter CO_2 CH_4 and N_2O fluxes on some natural and drained boreal peatlands. Biogeochemistry 44:163–186

Appelhans T, Detsch F (2015b) Unsupervised classification of Google maps imagery in R. doi:10.13140/RG.2.1.2092.8484

Appelhans T, Mwangomo E, Otte I, Detsch F, Nauss T, Hemp A (2015a) Eco-meteorological characteristics of the southern slopes of Kilimanjaro, Tanzania. Int J Climatol 36:3245–3258. doi:10.1002/joc.4552

Arias-Navarro C, Díaz-Pinés E, Kiese R, Rosenstock TS, Rufino MC, Stern D, Neufeldt H, Verchot LV, Butterbach-Bahl K (2013) Gas pooling: a sampling technique to overcome spatial heterogeneity of soil carbon dioxide and nitrous oxide fluxes. Soil Biol Biochem 67:20–23

Ball BC, Dobbie KE, Parker JP, Smith KA (1997) The influence of gas transport and porosity on methane oxidation in soils. Journal of Geophysical Research: Atmospheres (1984–2012) 102:23301–23308

Bender M, Conrad R (1994) Microbial oxidation of methane, NH_4^+ and carbon monoxide, and turnover of nitrous oxide and nitric oxide in soils. Biogeochemistry 27:97–112

Boeckx P, van Cleemput O, Villaralvo I (1997) Methane oxidation in soils with different textures and land use. Nutrient cycling in Agroecosystems 49:91–95

Booth MS, Stark JM, Rastetter E (2005) Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. Ecological monographs 75:139–157

Butterbach-Bahl K, Dannenmann M (2012) Soil carbon and nitrogen interactions and biosphere-atmosphere exchange of methane and nitrous oxide. In Recarbonization of the Biosphere-Ecosystems and the global Carbon Cycle Ed Lal R, Lorenz K, Hüttl RF, Schneider BU, von Braun J pp 429–443 Springer, Netherlands

Butterbach-Bahl K, Papen H (2002) Four years continuous record of CH_4-exchange between the atmosphere and untreated and limed soil of a N-saturated spruce and beech forest ecosystem in Germany. Plant Soil 240:77–90

Butterbach-Bahl K, Gundersen P, Ambus P, Augustin J, Beier C, Boeckx P, Dannenmann M, Sanchez Gimeno B, Ibrom A, Kiese R, Kitzler B, Rees RM, Smith KA, Stevens C, Vesala T, Zechmeister-Boltenstern S (2011) Nitrogen processes in terrestrial ecosystems. The European nitrogen assessment: sources, effects and policy perspectives. Cambridge University Press, Cambridge, pp. 99–125

Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S (2013) Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philosophical Transactions of the Royal Society B: Biological Sciences 368:20130122

Buytaert W, Cuesta-Camacho F, Tobón C (2011) Potential impacts of climate change on the environmental services of humid tropical alpine regions. Glob Ecol Biogeogr 20:19–33

Castro MS, Steudler PA, Melillo JM, Aber JD, Bowden RD (1995) Factors controlling atmospheric methane consumption by temperate forest soils. Glob Biogeochem Cycles 9:1–10

Chapman SK, Langley JA, Hart SC, Koch GW (2006) Plants actively control nitrogen cycling: uncorking the microbial bottleneck. New Phytol 169:27–34

Clein JS, Schimel JP (1995) Microbial activity of tundra and taiga soils at sub-zero temperatures. Soil Biol Biochem 27:1231–1234

Conrad R (1996) Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiological reviews 60:609–640

Cookson W, Cornforth I, Rowarth J (2002) Winter soil temperature (2–15 °C) effects on nitrogen transformations in clover green manure amended or unamended soils: a laboratory and field study. Soil Biol Biochem 34:1401–1415

Dannenmann M, Gasche R, Ledebuhr A, Papen H (2006) Effects of forest management on soil N cycling in beech forests stocking on calcareous soils. Plant Soil 287:279–300

Dannenmann M, Gasche R, Papen H (2007) Nitrogen turnover and N_2O production in the forest floor of beech stands as influenced by forest management. J Plant Nutr Soil Sci 170:134–144

Dannenmann M, Simon J, Gasche R, Holst J, Naumann PS, Kögel-Knaber I, Knicker H, Mayer H, Schloter M, Pena R, Polle A, Rennenberg H, Papen H (2009) Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. Soil Biol Biochem 41:1622–1631

Davidson EA, Hart SC, Shanks CA, Firestone MK (1991) Measuring gross nitrogen mineralization, and nitrification by ^{15}N isotopic pool dilution in intact soil cores. Journal of. Soil Sci 42:335–349

Davidson EA, Hart SC, Firestone MK (1992) Internal cycling of nitrate in soils of a mature coniferous forest. Ecology 73:1148–1156

Davidson E, Belk E, Boone RD (1998) Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Glob Chang Biol 4:217–227

De Bruijn AMG, Butterbach-Bahl K, Blagodatsky S, Grote R (2009) Model evaluation of different mechanisms driving

freeze–thaw N_2O emissions. Agric Ecosyst Environ 133: 196–207

Dentener F, Drevet J, Lamarque JF, Bey I, Eickhout AM, Fiore AM, Hauglaustaine D, Horowitz LW, Krol M, Kulshrestha UC, Lawrence M, Galy-Lacaux C, Rast S, Shindell D, Stevenson D, Van Noije T, Atherton C, Bell N, Bergmann D, Butler T, Cofala J, Collins B, Doherty R, Ellingsen K, Galloway J, Gauss M, Montanaro V, Müller JF, Pitari G, Rodriguez J, Sanderson M, Solmon F, Strahan S, Schultz M, Sudo K, Szopa S, Wild O (2006) Nitrogen and sulfur deposition on regional and global scales – a multimodel evaluation. Glob Biogeochem Cycles 20. doi:10.1029/2005GB002672

Downie C, Humphries DR, Wilcockson WH, Wilkinson P (1956) Geology of Kilimanjaro. Nature 178:828–830

Dutaur L, Verchot LV (2007) A global inventory of the soil CH_4 sink. Glob Biogeochem Cycles 21. doi:10.1029/2006GB002734

Ernakovich JG, Hopping KA, Berdanier AB, Simpson RT, Kachergis E, Steltzer H, Wallenstein MD (2014) Predicted responses of arctic and alpine ecosystems to altered seasonality under climate change. Glob Chang Biol 20:3256–3269

Geßler A, Jung K, Gasche R, Papen H, Heidenfelder A, Börner E, Metzler B, Augustin S, Hildebrand E, Rennenberg H (2005) Climate and forest management influence nitrogen balance of European beech forests: microbial N transformations and inorganic N net uptake capacity of mycorrhizal roots. Eur J For Res 124:95–111

Gulledge J, Schimel JP (2000) Controls on soil carbon dioxide and methane fluxes in a variety of taiga forest stands in interior Alaska. Ecosystems 3:269–282

Güsewell S (2004) N: P ratios in terrestrial plants: variation and functional significance. New Phytol 164:243–266

Hemp A (2006) Vegetation of Kilimanjaro: hidden endemics and missing bamboo. Afr J Ecol 44:305–328

Henry HAL (2007) Soil freeze–thaw cycle experiments: trends, methodological weaknesses and suggested improvements. Soil Biol Biochem 39:977–986

Hertel D, Leuschner C (2002) A comparison of four different fine root production estimates with ecosystem carbon balance data in a Fagus–Quercus mixed forest. Plant Soil 239:237–251

Hodge A, Robinson D, Fitter A (2000) Are microorganisms more effective than plants at competing for nitrogen? Trends in plant science 5:304–308

Holst J, Liu C, Yao Z, Brüggemann N, Zheng X, Giese M, Butterbach-Bahl K (2008) Fluxes of nitrous oxide, methane and carbon dioxide during freezing–thawing cycles in an inner Mongolian steppe. Plant Soil 308(1–2):105–117. doi:10.1007/s11104-008-9610-8

Jaeger CH III, Monson RK, Fisk MC, Schmidt SK (1999) Seasonal partitioning of nitrogen by plants and soil microorganisms in an alpine ecosystem. Ecology 80:1883–1891

Kielland K, Olson K, Ruess RW, Boone RD (2006) Contribution of winter processes to soil nitrogen flux in taiga forest ecosystems. Biogeochemistry 81:349–360

Kielland K, McFarland JW, Ruess RW, Olson K (2007) Rapid cycling of organic nitrogen in taiga Forest ecosystems. Ecosystems 10:360–368

Kiese R, Wochele S, Butterbach-Bahl K (2008) Site specific and regional estimates of methane uptake by tropical rainforest soils in north eastern Australia. Plant Soil 309:211–226

Kirkham D, Bartholomew WV (1954) Equations for following nutrient transformations in soil, utilizing tracer Data1. Soil Sci Soc Am J 18:33

Kurganova I, Lopes De Gerenyu V, Rozanova L, Sapronov D, Myakshina T, Kudeyarov V (2003) Annual and seasonal CO_2 fluxes from Russian southern taiga soils. Tellus B 55: 338–344

Kuzyakov Y, Blagodatskaya E (2015) Microbial hotspots and hot moments in soil. Concept & review. Soil Biology and Biochemistry 83:184–199. doi:10.1016/j.soilbio.2015.01.025

Kuzyakov Y, Gavrichkova O (2010) Review. Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. Glob Chang Biol 16:3386–3406. doi:10.1111/j.1365-2486.2010.02179.x

Kuzyakov Y, Xu X (2013) Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. The New Phytologist 198:656–669. doi:10.1111/nph.12235

Larsen KS, Jonasson S, Michelsen A (2002) Repeated freeze–thaw cycles and their effects on biological processes in two arctic ecosystem types. Appl Soil Ecol 21:187–195

Leuschner C, Hertel D, Coners H, Büttner V (2001) Root competition between beech and oak. A hypothesis. Oecologia 126: 276–284

Luo Y, Xuhui Z (2006) Soil respiration and the environment. Elsevier Academic press, Amsterdam, Boston

Luo GJ, Brüggemann N, Wolf B, Gasche R, Grote R, Butterbach-Bahl K (2012) Decadal variability of soil CO_2, NO, N_2O, and CH_4 fluxes at the Höglwald Forest, Germany. Biogeosciences 9:1741–1763. doi:10.5194/bg-9-1741-2012

Matzner E, Borken W (2008) Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review. Eur J Soil Sci 59:274–284. doi:10.1111/j.1365-2389.2007.00992.x

Mueller-Dombois D, Ellenberg H (1974) Aims and methods of vegetation ecology. Wiley, New York

Pabst H, Kühnel A, Kuzyakov Y (2013) Effect of land-use and elevation on microbial biomass and water extractable carbon in soils of Mt. Kilimanjaro ecosystems. Appl Soil Ecol 67: 10–19. doi:10.1016/j.apsoil.2013.02.006

Parkin T, Mosier A, Smith J, Ventera R, Johnson J, Reicosky D, Doyle G, McCarthy G, Baker J (2003) USDA-ARS GRACEnet Chamber-based Trace Gas Flux Measurement Protocol.afrsweb.usda.gov/SP2UserFiles/person/31831/2003GRACEnetTraceGasProtocol.pdf

Phillips RP, Finzi AC, Bernhardt ES (2011) Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecol Lett 14:187–194

Prescott CE (2010) Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 101:133–149

Raich JW, Schlesinger WH (1992) The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus B 44:81–99

Raich JW, Tufekciogul A (2000) Vegetation and soil respiration: correlations and controls. Biogeochemistry 48:71–90

Rennenberg H, Stoermer H, Weber P, Daum M, Papen H (2001) Competition of spruce trees for substrates of microbial N_2O - production and-emission in a forest ecosystem. Journal of applied botany 75:101–106

Rennenberg H, Dannenmann M, Gessler A, Kreuzwieser J, Simon J, Papen H (2009) Nitrogen balance in forest soils: nutritional limitation of plants under climate change stresses. Plant Biol 11:4–23

Rosenkranz P, Brüggemann N, Papen H, Xu Z, Butterbach-Bahl K (2005) N 2 O, NO and CH 4 exchange, and microbial N turnover over a Mediterranean pine forest soil. Biogeosci Discuss 2:673–702

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602

Schmidt SK, Nemergut DR, Miller AE, Freeman KR, King AJ, Seimon A (2009) Microbial activity and diversity during extreme freeze-thaw cycles in periglacial soils, 5400 m elevation, Cordillera Vilcanota, Perú. Extremophiles: life under extreme conditions 13:807–816. doi:10.1007/s00792–009–0268-9

Schütt M, Borken W, Spott O, Stange CF, Matzner E (2014) Temperature sensitivity of C and N mineralization in temperate forest soils at low temperatures. Soil Biol Biochem 69: 320–327

Shaver GR, Chapin FSIII (1991) Production: biomass relationships and element cycling in contrasting arctic vegetation types. Ecol Monogr 61:1–31

Silver WL, Herman DJ, Firestone MK (2001) Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. Ecology 82:2410–2416

Steinkamp R, Butterbach-Bahl K, Papen H (2001) Methane oxidation by soils of an N limited and N fertilized spruce forest in the black Forest, Germany. Soil Biol Biochem 33:145–153

Van Praag H, Sougnez - Remy S, Weissen F, Carletti G (1988) Root turnover in a beech and a spruce stand of the Belgian Ardennes. Plant and Soil 105:87–103

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

Verhagen FJM, Laanbroek HJ (1991) Competition for ammonium between nitrifying and heterotrophic bacteria in dual energy-limited chemostats. Appl Environ Microbiol 57:3255–3263

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. Ecol Appl 7:737–750

Weintraub MN, Schimel JP (2005) Nitrogen cycling and the spread of shrubs control changes in the carbon balance of arctic tundra ecosystems. Bioscience 55:408–415

Wolf B, Zheng X, Brüggemann N, Chen W, Dannenmann M, Han X, Sutton MA, Wu H, Yao Z, Butterbach-Bahl K (2010) Grazing-induced reduction of natural nitrous oxide release from continental steppe. Nature 464:881–884. doi:10.1038/nature08931

Wookey PA, Aerts R, Bardgett RA, Baptist F, Bråthen KA, Cornelissen JHC, Gough L, Hartley IP, Hopkins DW, Lavorel S, Shaver GR (2009) Ecosystem feedbacks and cascade processes. Understanding their role in the responses of Arctic and alpine ecosystems to environmental change. Glob Chang Biol 15:1153–1172. doi:10.1111/j.1365-2486.2008.01801.x

WRB (2014) World reference base for soil resources. International soil classification system for naming soils and creating legends for soil maps. Rome: Food and Agriculture Organization of the United Nations

Wu H, Dannenmann M, Wolf B, Han X, Zheng X, Butterbach-Bahl K (2012) Seasonality of soil microbial nitrogen turnover in continental steppe soils of Inner Mongolia. Ecosphere 3:1–18

Zech M (2006) Evidence for late Pleistocene climate changes from buried soils on the southern slopes of Mt. Kilimanjaro, Tanzania. Palaeogeogr Palaeoclimatol Palaeoecol 242:303–312

Zech M, Hörold C, Leiber-Sauheitl K, Kühnel A, Hemp A, Zech W (2014) Buried black soils on the slopes of Mt. Kilimanjaro as a regional carbon storage hotspot. CATENA 112:125–130