

# Shift in soil–plant nitrogen dynamics of an alpine–nival ecotone

Edith Huber · Wolfgang Wanek ·  
Michael Gottfried · Harald Pauli ·  
Peter Schweiger · Stefan K. Arndt · Karl Reiter ·  
Andreas Richter

Received: 25 February 2007 / Accepted: 5 September 2007 / Published online: 3 October 2007  
© Springer Science + Business Media B.V. 2007

**Abstract** We investigated the nitrogen (N) dynamics of an alpine–nival ecotone on Mt. Schrankogel, Tyrol, Austria, in relation to temperature. Natural abundance of  $^{15}\text{N}$  was used as a tool to elucidate differences in N cycling along an altitudinal transect ranging from 2,906 to 3,079 m, corresponding to a gradient in mean annual temperature of 2.4 °C. The amount of total soil N, of plant available N and soil C/N ratio decreased

significantly with increasing altitude, whereas soil pH increased. Soil  $\delta^{15}\text{N}$  decreased with increasing altitude from +2.2 to –2.1‰ and  $\delta^{15}\text{N}$  of plant tissues (roots and leaves) decreased from –3.7 to –5.5‰. The large shift in soil  $\delta^{15}\text{N}$  of 4.3‰ from the lowest to the highest site suggested substantial differences in N cycling in alpine and nival ecosystems in the alpine nival ecotone investigated. We concluded that N cycling at the alpine–nival ecotone is likely to be controlled by various factors: temperature, soil age and development, atmospheric N deposition and plant competition. Our results furthermore demonstrate that the alpine–nival ecotone may serve as a sensitive indicator of global change.

Responsible Editor: Euan James

E. Huber · W. Wanek · A. Richter (✉)  
Department of Chemical Ecology and Ecosystem Research,  
University of Vienna,  
Althanstraße 14,  
1090 Vienna, Austria  
e-mail: Andreas.Richter@univie.ac.at

E. Huber · S. K. Arndt · A. Richter  
School of Forest and Ecosystem Science,  
The University of Melbourne,  
Water St., Creswick,  
Victoria 3363, Australia

M. Gottfried · H. Pauli · K. Reiter  
Department of Conservation Biology,  
Vegetation and Landscape Ecology, University of Vienna,  
Althanstraße 14,  
1090 Vienna, Austria

P. Schweiger  
Department of Forest and Soil Science,  
University of Natural Resources and Applied Life Sciences,  
Peter Jordan Straße 82,  
1190 Vienna, Austria

**Keywords** Climate change · High mountains · Mineralization · Nitrification · Stable isotopes · Temperature · Radiocarbon

## Introduction

Average global surface temperatures have increased by  $0.6 \pm 0.2$  °C over the last century. The trend of increasing annual temperatures is expected to continue with predictions of a rise of global mean air temperatures of 1.8 to 4.0 °C by 2100 (Metz et al. 2007). Some natural ecosystems will be severely affected by climate change due to their limited adaptive capacity (Adger et al. 2007; Watson et al. 1997). In particular,

alpine ecosystems are vulnerable to climate change because of their isolation and fragmentation and the existence of multiple stresses (Watson et al. 1997). Effects of climate warming on alpine vegetation have already been observed. For example, plant species have migrated upwards, increasing the number of species on high altitude summits (Grabherr et al. 1994; Pauli et al. 2001). Such changes in ecosystem composition, structure and diversity are apparent in high elevation ecosystems in Europe and have been attributed to warmer winter and summer temperatures, and longer growing seasons (Klanderud and Birks 2003; Kullman 2002; Moiseev and Shiyatov 2003; Virtanen 2003; Walther et al. 2005).

Vegetation changes in high altitude ecosystems as a response to climate change can also be associated with concomitant changes in soil processes as an altered temperature regime can affect the turnover of soil nutrients and plant community structures (Chapin et al. 1995). Soil enzymatic activities decrease with increasing altitude and decreasing temperature (Schinner 1982), and rates of N mineralization also decrease (Jacot et al. 2000b; Sveinbjornsson et al. 1995). Increased temperatures can therefore profoundly influence soil processes in cold environments, by increasing nutrient availability (Grogan and Chapin 2000; Michelsen et al. 1996) and enhancing rates of N mineralization (Hartley et al. 1999; Jonasson et al. 1999; Shaw and Harte 2001).

Although many studies have examined the effects of increasing temperature on nutrient cycling in arctic ecosystems (Grogan and Chapin 2000; Hobbie 1996; Jonasson et al. 2004; Jonasson et al. 1993; Schmidt et al. 2002; van Heerwaarden et al. 2003), only few have addressed nutrient transformations in high altitude ecosystems *in-situ* along natural temperature gradients (Jacot et al. 2000a; Morecroft et al. 1992; Sveinbjornsson et al. 1995) and their susceptibility to climate change. The aim of our study was to investigate the N dynamics of an alpine–nival ecotone on the basis of a relatively short altitudinal gradient (173 m) that was linked to a difference in mean annual temperatures of 2.4 °C. Due to the sensitivity of the prevalent vegetation to environmental conditions, the alpine–nival ecotone is ideally suited to study climate change effects (Gottfried et al. 1998) We hypothesized that lower air and soil temperatures due to increasing altitude will affect concentrations of plant available N in the soil and influence rates of soil N cycling.

Natural abundance of the stable isotope  $^{15}\text{N}$  was used as a tool to study ecosystem processes as it acts as an integrator of the long-term ecosystem N balance (Högberg 1997). We expected that not only temperature but also other factors such as developmental stage and stability of the substrate influence N isotope signatures of soils and plants. In addition, roots were investigated for mycorrhizal colonisation and a complementary laboratory experiment was conducted to investigate the temperature sensitivity of two vital processes in the soil, N mineralization and nitrification.

## Material and methods

### Study area

The study was conducted at Mount Schrankogel, Stubai Alps, Tyrol, Austria (11°05'58"E, 47°02'41" N; 3497 m) in August 1999 and 2000. The investigation area was located on a southwest slope between 2,900 m and 3,100 m a.s.l. The underlying parent material of the area around Mt. Schrankogel consists of siliceous material, mostly amphibolites (Patzelt 1976). Prevalent soil types under the alpine *Carex curvula* grassland and the patchy alpine–nival plant assemblages are alpine ranker, turf brown soils and alpine raw soils (leptosols and cambisols), respectively (Dullinger 1998). On the southwest ridge and slope, the closed alpine *C. curvula* grassland disintegrates into sward fragments at around 2,900 m and is finally replaced by nival rock and scree vegetation which is dominated by *Androsace alpina*, *Poa laxa* and *Saxifraga bryoides* (Gottfried et al. 1998; Pauli et al. 1999). This transition zone is referred to as alpine–nival ecotone (Gottfried et al. 1999). A vegetation classification by (Pauli et al. 1999) identified 14 vascular plant assemblages belonging to two vegetation types: alpine grassland (*Caricion curvulae*) and open, patchy nival plant assemblages (*Androsacion alpinae*). Closed alpine *C. curvula* grassland avoids unstable habitats with high cover of scree whereas nival vegetation establishes not only in rocky habitats but also on scree (Pauli et al. 1999).

### Study plots

Within the study area, five plots of 3×3 m were selected along an altitudinal transect (2,906 to

3,079 m a.s.l.). Vascular plant assemblages at the chosen altitudes are shown in Table 1. The study plots on Mt. Schrankogel were located in the immediate vicinity of permanent plots as they had been established and described previously (Gottfried et al. 1998). Since 1997, temperature loggers (Tinytag logger, Gemini Data Loggers, UK) have measured the air or snow temperature at 1–3 cm above the soil surface which in terms of alpine plant life represents a key microclimatic zone (Gottfried et al. 1998). Mean annual temperatures for a period of three years are presented in Table 2 for each study plot. Temperature data clearly show that the altitudinal gradient is linked to a temperature gradient. (Gottfried et al. 1998) found on average an annual mean temperature lapse rate of 0.95°C per 100 m altitudinal increase between 2,900 and 3,500 m at Mt. Schrankogel.

### Soil and plant sampling

Soils were collected in August 1999 and 2000, while plants were sampled in August 2000. Five replicate soil samples were taken from the upper 10 cm of the soil profile of each 3×3 m study plot. The samples were put into cold storage (4 °C) immediately after collection and transported to Vienna. Soils were

**Table 1** Altitude (meter above sea level) and description of plant assemblages of the study plots on Mt. Schrankogel, Tyrol, Austria

Site	Altitude (m)	Vascular plant assemblage
1	2,906	Closed alpine sward ( <i>Caricetum curvulae</i> ), closed vegetation
2	2,944	Pioneer sward vegetation, transition between closed and open vegetation
3	2,987	Alpine–nival cushion plant community on siliceous scree ( <i>Androsacetum alpinae</i> ), vegetation in patches
4	3,030	Alpine–nival cushion plant community on siliceous scree ( <i>Androsacetum alpinae</i> ), vegetation in patches
5	3,079	Alpine–nival cushion plant community on siliceous scree ( <i>Androsacetum alpinae</i> ), vegetation in patches

sieved (<2 mm) and after the removal of fine roots stored at 4 °C. Extraction with 1 M KCl for solute analysis was conducted within a week after collection. Aliquots of soil samples were oven-dried at 60 °C for 2 days before isotope analysis. Above- and below-ground plant parts were collected from 19 plant species (Table 3). Plant species were chosen according to their abundance at this altitudinal transect, focusing on the major species and on those that occurred at least in two different altitudinal plots at the study site. Samples were collected from within the 3×3 m plots where soil samples have been taken. Three replicate plants were sampled at each site as only a few individuals of each species were present in the plots. Plants were separated into roots and shoots and oven-dried at 60 °C.

### Soil pH, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, dissolved organic nitrogen

Soil pH (0.01 M CaCl<sub>2</sub>) was determined from sieved soil samples (<2 mm) using a soil:solution ratio of 1:2.5. Nitrate was analysed in soil water extracts (1:10, w/v) by chemically suppressed ion chromatography (DX 500; Dionex, Vienna, Austria). Anions were separated on an anion exchange column (AS11; 250×4 mm i. d., Dionex) using a linear KOH gradient (2 to 40 mM in 7 min.). Ammonium and α-amino-N concentrations were determined in aliquots of 1 M KCl extracts (1:10, w/v) of the soils using colorimetric techniques in a microtiterplate reader photometer (Tecan Rainbow, Austria). Quantification of ammonium was carried out using the indophenol method (Kandeler and Gerber 1988) and of α-amino-N using the ninhydrin reaction (Amato and Ladd 1988). For the latter method, any interfering ammonium was removed by increasing the pH of extracts with MgO resulting in gaseous loss of ammonium as ammonia. From hereon we refer to the ninhydrin-reactive N as dissolved organic nitrogen (DON).

### Nitrogen and carbon isotope composition

Dried soil and plant samples were ground to a fine powder in a ball mill (Retsch MM2; Retsch, Vienna, Austria), weighed into tin capsules and analysed by continuous-flow gas isotope ratio mass spectrometry (IRMS). The IRMS system consisted of an elemental analyser (EA 1110, CE Instruments, Milan, Italy) coupled via a ConFlo II interface (Finnigan MAT,

**Table 2** Characteristics (pH, C–N ratios, total N, total C and  $\delta^{13}\text{C}$ ) of top soils (0–10 cm) of the five study plots on Mt. Schrankogel, Tyrol, Austria

Site	MAT (°C)	pH (H <sub>2</sub> O)	C–N ratio	Total N (% DM)	Total C (% DM)	$\delta^{13}\text{C}$ (‰)
1	1.7	4.64 (0.08)	14.34 (0.58)	0.19 (0.01)	2.71 (0.14)	–24.43 (0.10)
2	1.6	5.26 (0.06)	12.40 (0.28)	0.10 (0.03)	1.25 (0.35)	–24.52 (0.18)
3	0.4	5.31 (0.05)	11.55 (0.22)	0.07 (0.01)	0.80 (0.15)	–24.30 (0.19)
4	0.2	5.34 (0.09)	12.61 (0.63)	0.08 (0.02)	0.97 (0.23)	–24.56 (0.20)
5	–0.7	5.51 (0.05)	12.31 (0.39)	0.05 (0.01)	0.62 (0.10)	–24.72 (0.16)

Mean annual temperatures (MAT) are given for the period of mid 1997 to mid 2000. Values are means  $\pm$  SE ( $n=5$ ), except for MAT.

Bremen, Germany) to the gas isotope ratio mass spectrometer (Delta<sup>PLUS</sup>, Finnigan MAT). Reference gas (high purity CO<sub>2</sub> and N<sub>2</sub> gas; AGA, Vienna, Austria) was calibrated to the Vienna-Pee Dee Belemnite (V-PDB) standard using IAEA-CH-6 and IAEA-CH-7, and to the atmospheric N<sub>2</sub> standard (at-air) using IAEA-NO-3, IAEA-N-1 and -2 reference material (International Atomic Energy Agency,

Vienna, Austria). The abundance of C and N isotopes of each sample was determined in a single run, and N<sub>2</sub> and CO<sub>2</sub> reference gases were run with each analysis. The natural abundance of <sup>15</sup>N and <sup>13</sup>C was calculated as follows:

$$\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 \text{ [‰ vs at - air]}$$

$$\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 \text{ [‰ vs V - PDB]}$$

**Table 3** Plant species collected on Mt. Schrankogel, Tyrol, Austria for analysis of natural abundance of <sup>15</sup>N

Species name	Family	Study site
<i>Erigeron uniflorus</i> L.	Asteraceae	4
<i>Leucanthemopsis alpina</i> (L.) Heywood	Asteraceae	1, 2, 3, 4, 5
<i>Cerastium uniflorum</i> Clairv.	Caryophyllaceae	2, 3, 4, 5
<i>Minuartia sedoides</i> (L.) Hiern	Caryophyllaceae	1, 2
<i>Silene exscapa</i> All.	Caryophyllaceae	1, 2, 3, 4
<i>Carex curvula</i> All.	Cyperaceae	1
<i>Gentiana bavarica</i> L.	Gentianaceae	3, 4
<i>Luzula spicata</i> (L.) DC.	Juncaceae	1, 2, 3, 4, 5
<i>Avenula versicolor</i> (Vill.) M.Lainz	Poaceae	1
<i>Festuca intercedens</i> (Hack.) Lüdi ex Bech.	Poaceae	1
<i>Oreochloa disticha</i> (Wulfen) Link	Poaceae	1, 2
<i>Poa alpina</i> L.	Poaceae	2, 3, 4
<i>Poa laxa</i> Haenke	Poaceae	1, 2, 3, 4, 5
<i>Androsace alpina</i> (L.) Lam.	Primulaceae	1, 2, 3
<i>Primula glutinosa</i> Wulfen	Primulaceae	1, 2
<i>Ranunculus glacialis</i> L.	Ranunculaceae	4, 5
<i>Saxifraga bryoides</i> L.	Saxifragaceae	1, 2, 3, 4, 5
<i>Saxifraga exarata</i> Vill.	Saxifragaceae	5
<i>Veronica alpina</i> L.	Scrophulariaceae	2, 3, 4

Study plots are described in Table 1.

where R is the ratio of <sup>15</sup>N/<sup>14</sup>N for nitrogen and <sup>13</sup>C/<sup>12</sup>C for carbon isotope abundance. The precision of isotope measurements of a laboratory standard was 0.15‰ for  $\delta^{15}\text{N}$  and 0.10‰ for  $\delta^{13}\text{C}$ .

#### Gross mineralization and gross nitrification

Gross mineralization and gross nitrification were determined in the replicated soil samples that were collected from the 3×3 m plots at three altitudes (2,906 m, 2,944 m and 3,030 m) in 1999 and 2000, and incubation temperature was varied between 0 and 24 °C. Rates of gross nitrogen transformations were determined by <sup>15</sup>N pool-dilution technique. Duplicate samples (2 g) of sieved soil (<2 mm) were transferred into polyethylene vials and mixed with 0.5 ml of a 0.25 mM (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> or 0.5 ml of a 0.5 mM <sup>15</sup>KNO<sub>3</sub> solution for the mineralization and nitrification assays, respectively. Mineralization and nitrification assays were stopped after 4 h in half of the samples and in the second half after 20 h by extracting with 14 ml of 1 M KCl for 4 h. In order to remove interfering organic nitrogen from the nitrification assays, samples were treated with 30% H<sub>2</sub>O<sub>2</sub> and concentrated H<sub>2</sub>SO<sub>4</sub> in a UV digester (60 min). In this reaction, organic nitrogen was decomposed and the ammonium released was removed by increasing the pH >9.5 through addition of

32% NaOH and shaking on a horizontal shaker for 2 days. By adding Devarda reagent, nitrate was then converted to ammonium which was collected by micro-diffusion in acid traps at 25 °C for 48 h (Khan et al. 1998). Acid traps were made of discs of ash-free filter paper amended with 15 µl 2.5 M KHSO<sub>4</sub> and coated in strips of teflon tape. Mineralization samples were treated with MgO to increase the solution pH to >9.5 and the ammonia released into the headspace was collected as above. Thereafter, acid traps were dried over concentrated H<sub>2</sub>SO<sub>4</sub>, the filter papers were removed from the Teflon coating, transferred into tin capsules and the amount of <sup>15</sup>N and total N measured by IRMS.

Rates of gross mineralization and gross nitrification were calculated using the equations described in (Barrett and Burke 2000):

$$m = \frac{M_0 - M}{t} \times \frac{\log H_0 M / H M_0}{\log M_0 / M}$$

$$n = \frac{M_0 - M}{t} \times \frac{\log H_0 M / H M_0}{\log M_0 / M}$$

$m$	gross mineralization in mg kg <sup>-1</sup> d <sup>-1</sup>
$n$	gross nitrification in mg kg <sup>-1</sup> d <sup>-1</sup>
$H_0, H$	<sup>15</sup> N g soil <sup>-1</sup> at time $t=0$ and $t=t$ respectively ( $t=20$ h)
$M_0, M$	total N ( <sup>14</sup> N + <sup>15</sup> N g soil <sup>-1</sup> ) at time $t=0$ and $t=t$ respectively ( $t=20$ h)

#### Determination of mycorrhizal root colonisation

For assessment of root colonisation by arbuscular mycorrhizal (AM) and dark septate (DS) fungi, roots were cleared in 10% KOH at 90 °C for 30 min and thereafter stained employing the ink/vinegar method (Vierheilig et al. 2005). Randomly selected root pieces (three per plant) from species collected in all five study plots were mounted on microscope slides and the percentage of root length colonised by the respective fungi was assessed under a microscope at 100× magnification (McGonigle et al. 1990). Colonisation data are expressed as the percentage of total root intersects examined that contain fungal structures (= % root length colonised).

#### Radiocarbon determination

Radiocarbon measurements of soil organic matter were made by accelerator mass spectrometry (AMS)

at the Vienna Environmental Research Accelerator (VERA #2502–#2504). For this analysis, the humic acid fraction was used. The <sup>14</sup>C data (corrected for isotopic fractionation) are presented as pMC (percent Modern Carbon) and, where pMC values were <100, as radiocarbon age (years BP) (Stuiver and Polach 1977).

#### Statistical analysis

Statistical analysis was carried out by polynomial regression analysis based on first and second order models using Statgraphics Centurion XV software (Statistical Graphics Corp.).

## Results

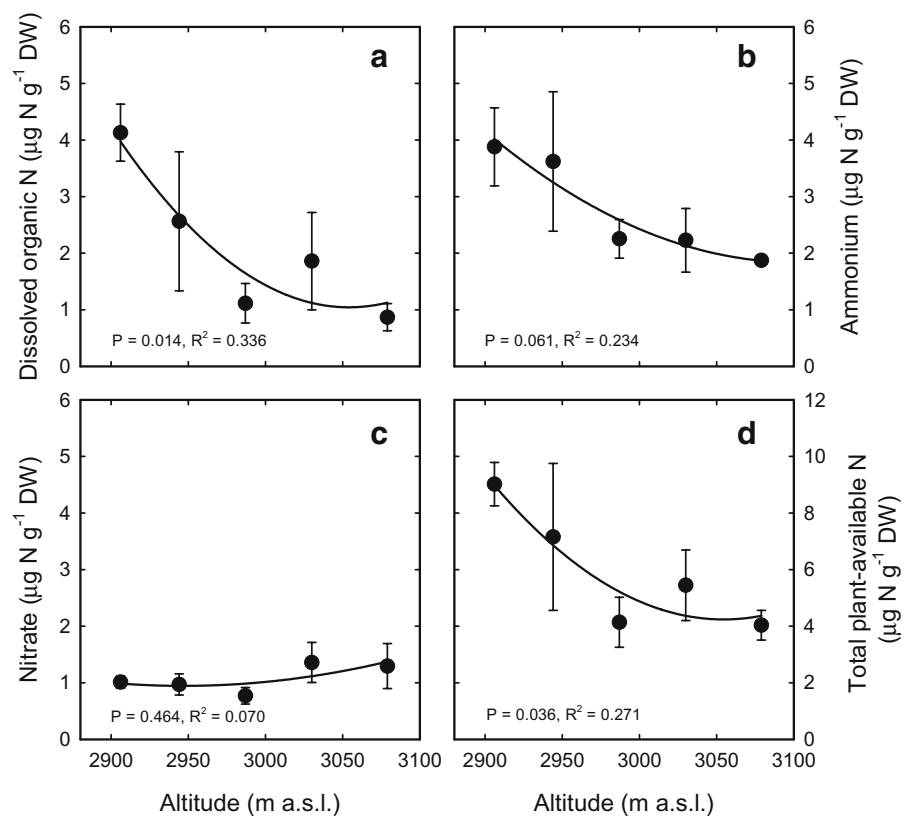
### Temperature, soil pH, soil N concentration and C isotope composition

With increasing altitude mean annual air temperature on Mt. Schrankogel, measured at 1–3 cm above soil surface, decreased from 1.7 °C at the lowest elevation (2,906 m) to -0.7 °C at the highest elevation (3,079 m) (Table 2). The temperature difference between the lowest and highest altitude was therefore 2.4 °C for the years 1997–2000. Soil pH increased from 4.6 to 5.5 with altitude (Table 2). Soil C–N ratio, total N and total C decreased along the altitudinal gradient (Table 2), whereby the lowest site (2,906 m) was always markedly different from the sites at higher altitudes. Soil δ<sup>13</sup>C did not change with increasing elevation (Table 2).

### <sup>15</sup>N natural abundance of soil and plant material

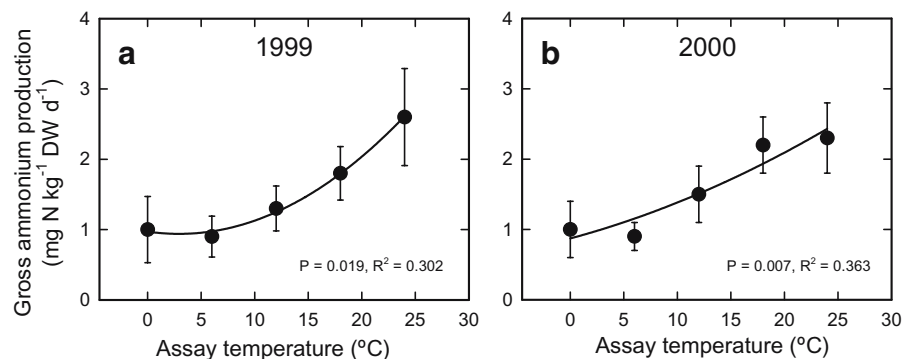
The δ<sup>15</sup>N signatures of soil (polynomial regression analysis, second order model;  $P < 0.001$ ) and plant material ( $P < 0.001$ ) decreased significantly with elevation and were more positive at the lowest altitude (Fig. 1). The biggest difference of soil δ<sup>15</sup>N was between the site at 2,906 m and at 3,030 m, where δ<sup>15</sup>N values decreased by 5.5‰ (from +2.15‰ to -3.34‰). Soil pH and δ<sup>15</sup>N of soil exhibited a significant negative linear relationship (Fig. 2) where δ<sup>15</sup>N of soils decreased ( $R^2 = 0.57$ ,  $P < 0.001$ ) with decreasing soil acidity. δ<sup>15</sup>N values of soils were always more positive than those of plant tissues

**Fig. 1** Relationship between soil concentrations of dissolved organic N (a), ammonium (b), nitrate (c) and total plant-available N (d) and altitude at Mt. Schrankogel, Austria. Error bars represent standard errors ( $n=5-15$ ). Results of a polynomial regression analysis (second order model) are given



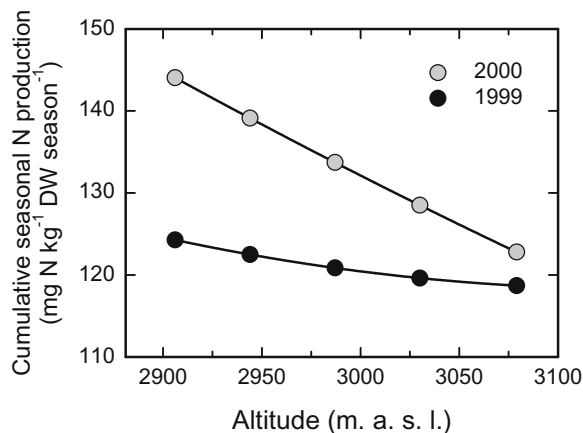
whereas  $\delta^{15}\text{N}$  signatures of leaves and roots were similar (Fig. 1). The shift in  $\delta^{15}\text{N}$  values along the altitudinal gradient was more pronounced in soils (5.5‰) than in plants where  $\delta^{15}\text{N}$  values only decreased by about 2‰. The four plant species that were collected across the whole altitudinal transect showed similar, significant negative relationships between  $\delta^{15}\text{N}$  and altitude. The trend was more pronounced for *Leucanthemopsis alpina* ( $P < 0.001$ ) and *Luzula spicata* ( $P < 0.001$ ) than for *P. laxa* ( $P = 0.023$ ) and *S. bryoides* ( $P = 0.065$ ) (3.0 to 3.5‰ versus 1.5 to 1.0‰).

**Fig. 2** Relationship between assay temperature and gross mineralization in top soils collected from an alpine–nival ecotone at Mt. Schrankogel, Austria, (a) in 1999 and (b) in 2000. Error bars represent standard errors ( $n=9$ ). Results of a polynomial regression analysis (second order model) are given



#### Mycorrhizal root colonisation

*L. alpina* from all five sites was heavily colonised by arbuscular mycorrhizal (AM) fungi (up to 80%), and only to a much lesser extent by dark septate (DS) fungi (between 0 to 10%). *L. spicata* roots contained no AM fungal structures, while root colonisation with DS fungi varied between 0 and 100% of total root length. *P. laxa* showed low levels of AM (0 to 15%) and highly variable levels of colonisation with DS fungi (0 to 65%) along the altitudinal transect. Root colonisation of *S. bryoides* ranged from 0 to 45% by



**Fig. 3** Modelled rates of gross mineralization for a growing season (May to August) at different altitudes at Mt. Schrankogel, Austria. Cumulative rates of  $\text{NH}_4^+$  production were calculated using the temperature-mineralisation relationships derived from Fig. 2 and mean monthly temperatures during May to August in 1999 and 2000

AM, and <5 to 100% by DS fungi. For all species, neither AM nor DS fungal root colonisation was correlated with altitude ( $P > 0.1$ ).

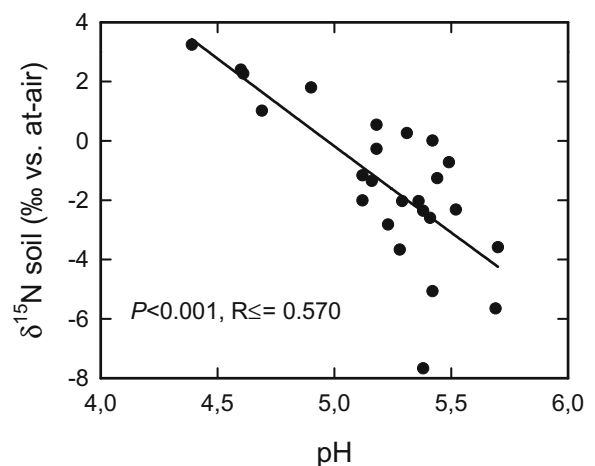
#### Plant available forms of N in soils

The sum of plant available N (i.e., ammonium, nitrate and DON) in soil extracts decreased with increasing altitude and concentrations at 2,906 m differed significantly from those at 2,987 m and 3,079 m (Fig. 3, polynomial regression analysis, second order model;  $P < 0.036$ ). A closer view on the fractions of available N in the soil extracts showed that the concentrations of ammonium ( $P = 0.061$ ) and of DON ( $P = 0.014$ ) decreased with altitude, whereas soil nitrate concentration did not change significantly with altitude ( $P = 0.464$ ). Consequently, the relative contribution of each form of N to the sum of available nitrogen changed with increasing altitude. At 2,906 m, ammonium, DON and nitrate made up for 43%, 46% and 11% of soluble N in the soil, respectively. At 3,079 m, the contribution of ammonium to plant available N in the soil was similar (46%), however, that of DON decreased to 22% and the contribution of nitrate increased to 32%.

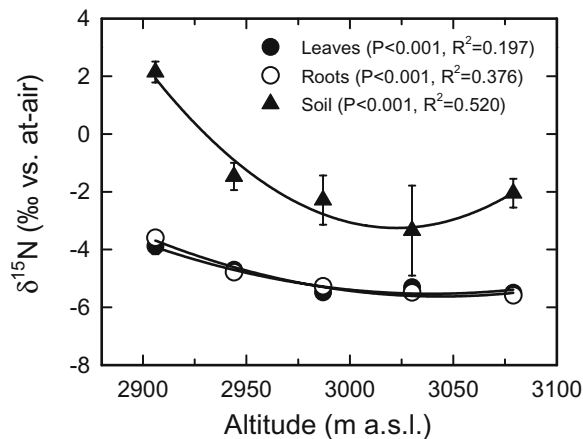
#### Rates of gross mineralization and nitrification – measured and calculated

We found no significant differences in the temperature sensitivity of gross transformation rates between

high and low altitude soils and therefore combined the data for Fig. 4. Rates of gross mineralization increased significantly from 0 °C to 24 °C as presented in Fig. 4a,b (polynomial regression analysis, second order model;  $P = 0.019$  and  $0.007$  for 1999 and 2000). The amounts of ammonium produced increased from  $1.03 \text{ mg N kg}^{-1} \text{ DW d}^{-1}$  at 0 °C to  $2.56 \text{ mg N kg}^{-1} \text{ DW d}^{-1}$  at 24 °C in 1999 and from  $0.98$  to  $2.30 \text{ mg N kg}^{-1} \text{ DW d}^{-1}$  in 2000. Rates of gross nitrification are not shown as they were low ( $< 0.4 \text{ mg N kg}^{-1} \text{ DW}$ ) and since the data did not indicate any temperature dependency of nitrate production. We used the results of the incubation experiment to model cumulative nitrogen release by gross mineralization in 1999 and 2000 (Fig. 5). Total nitrogen release during the growing season (May to August) was calculated by relating the rates of gross mineralization from the laboratory experiment to mean monthly air temperatures (measured at 1–3 cm above ground) in 1999 and 2000. The data showed that with increasing altitude cumulative seasonal N production decreased from 124 (144)  $\text{mg N kg}^{-1} \text{ DW season}^{-1}$  to 119 (123)  $\text{mg N kg}^{-1} \text{ DW season}^{-1}$  in the year 1999 (2000). Calculated in this way, seasonal N production was higher in 2000 than in 1999 due to a steeper gradient of mean annual temperatures between the lowest and highest altitude (slope  $k$  of quadratic regression was  $-0.2$  in 1999 and  $-0.8$  in 2000).



**Fig. 4** Relationship between soil pH and  $\delta^{15}\text{N}$  of soils from different altitudes between 2,906 m and 3,079 m at Mt. Schrankogel, Austria ( $n = 25$ ). Results of a polynomial regression analysis (second order model) are given



**Fig. 5** Relationship between  $\delta^{15}\text{N}$  signatures of top soils (0–10 cm),  $\delta^{15}\text{N}$  of leaves and roots of plant species and altitude at Mt. Schrankogel (see Table 3 for species list). Mean vegetation  $\delta^{15}\text{N}$  values of above- and belowground plant material were calculated from single plant species after weighing by species cover on the respective plots. Error bars represent standard errors ( $n=5\text{--}36$ ). Results of a polynomial regression analysis (second order model) are given

#### Radiocarbon analysis of humic fraction

$^{14}\text{C}$ -dating of the humic acid fraction of soils from three different altitudes showed that soils from the lowest altitude were  $665 \pm 30$  years old ( $92.1 \pm 0.4$  pMC) whereas humic acids from higher altitudes were less than 50 years old ( $105.2 \pm 0.5$  and  $101.6 \pm 0.6$  pMC for site 2 and 4, respectively).

#### Discussion

The N dynamics in the alpine–nival ecotone on Mt. Schrankogel clearly changed as mean annual air temperatures declined with increasing altitude. As hypothesized, lower temperatures reduced rates of ecosystem N turnover and N accumulation. This resulted in lower amounts of available and total N in soils at our higher altitude study plots (Table 2 and Fig. 1). A decline of soil organic matter with increasing altitude was also reflected by decreasing concentrations of total C in soil that led to a narrowing of C:N ratios with increasing altitude (Table 2). In vivo measurements of gross ammonium production at various temperatures (0–24 °C) supported our findings showing a reduction of rates of mineralization with decreasing temperature (Fig. 2). Interpolation of these numbers to overall seasonal

production of N yet again demonstrated lower rates of mineralization at higher altitudes where temperatures are lower (Fig. 3). Our data are in accordance with numerous studies in arctic and alpine ecosystems that reported a strong relationship between temperature and rates of N mineralization (e.g. (Hartley et al. 1999; Hobbie 1996; Jonasson et al. 1993; Nadelhoffer et al. 1991; Peterjohn et al. 1994).

Changes in rates of soil N turnover along the altitudinal gradient on Mt. Schrankogel influenced the availability of the different forms of N in the surface soil (Fig. 1). The concentrations of ammonium and DON in soils decreased with increasing altitude reflecting decreasing rates of N mineralization with altitude. In contrast, the concentrations of nitrate increased slightly with increasing altitude, possibly due to the insensitivity of nitrification to temperature shown in this study when compared with N mineralization.

In this context, it is important to acknowledge that temperature also influences plant assemblages and cover which in turn affect soil N cycling through litter quality and quantity (Bowman et al. 2004; Steltzer and Bowman 1998). The influence of plants on N dynamics potentially has important consequences for soil processes if climate warming alters the structure and composition of plant communities (Hobbie 1996). In addition, in such a scenario temperature may influence plant N uptake directly via increased productivity (Atkin and Cummins 1994; Garnett and Smethurst 1999; Volder et al. 2000). Moreover, soil microbial community composition (which itself is tightly interconnected with plant species composition) may play a crucial role in driving the cycling of N.

Although natural abundance of  $^{15}\text{N}$  does not allow a clear assessment of mechanisms or a quantification of process rates or process identity (Handley and Raven 1992) it provides a general understanding of underlying ecosystem processes. In our study, soil  $\delta^{15}\text{N}$  and pH were strongly correlated showing that soils with more acidic soil pH (as found in lower altitudes) had more positive  $\delta^{15}\text{N}$  values. Older soils usually have more positive  $\delta^{15}\text{N}$  signatures due to longer microbial processing (Kramer et al. 2003) and greater cumulative losses of  $^{15}\text{N}$  depleted N such as nitrate and nitrous oxide over an extended period of time (Brenner et al. 2001; Nadelhoffer and Fry 1994). Further, more developed soils usually have more acidic pH values as soil pH decreases with age (Cox and Whelan 2000; de Kovel et al. 2000; Munroe and



Bockheim 2001) and soil development (Berendse et al. 1998). This relationship and results from the  $^{14}\text{C}$ -dating of the humic acid fraction of soils from three different altitudes suggest that not only temperature influenced N cycling in the alpine–nival ecotone but also soil age and developmental stage. Indeed, soils under closed alpine grassland (alpine ranker and turf brown soils) are of a different type than soils under patchy nival plant assemblages (alpine raw soils) (Dullinger 1998), supporting the findings that soils at higher altitudes are younger and less well developed.

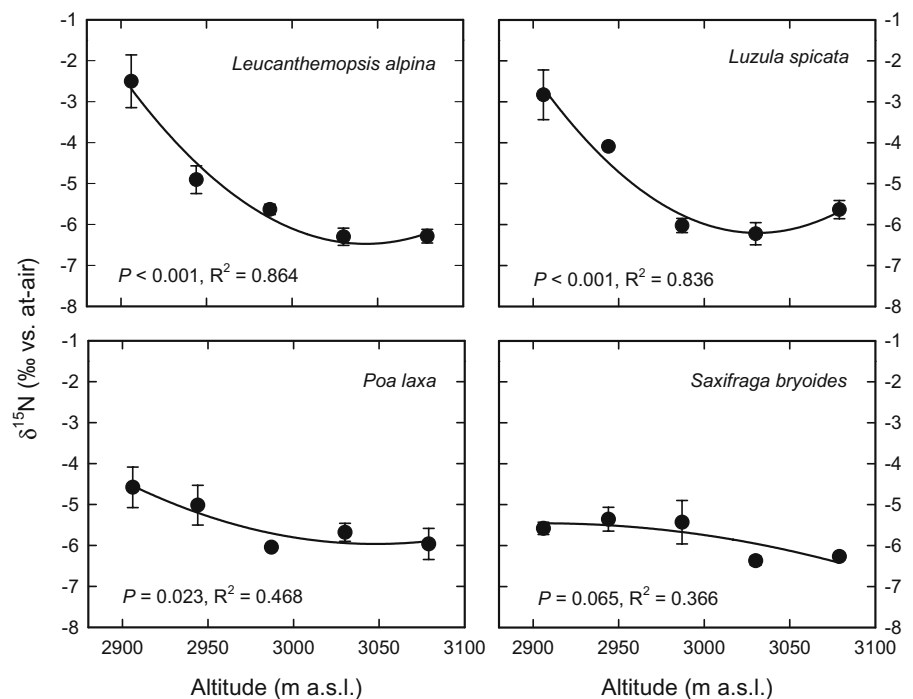
Substrate stability and duration of snow cover most likely also contribute to differences in soil development within the alpine–nival ecotone. Species in the nival zone can tolerate unstable scree whereas alpine species such as *C. curvula* preferably grow on more stable substrate (Pauli et al. 1999). Unstable habitats are subject to greater erosive forces leading to greater ecosystem N losses. Plant species in the nival zone on Mt. Schrankogel experience an even shorter vegetation period than species in the alpine zone owing to longer-lasting snow cover until the summer months (Gottfried et al. 2002). This also slows N cycling at higher altitudes.

Natural abundance of  $^{15}\text{N}$  in plant species decreased with increasing altitude (Fig. 6) in a similar way as soil  $\delta^{15}\text{N}$  which is expected as soil N and plant N nutrition are tightly connected. Various factors control plant  $\delta^{15}\text{N}$ ,

such as the isotopic composition of N sources and  $^{15}\text{N}$  discrimination during plant N acquisition, both of which are affected by microbial colonization of roots, by growth N demand and competition, and by nitrogen form. Internal N cycling in plants did not affect plant  $\delta^{15}\text{N}$  values as leaves and roots had similar  $\delta^{15}\text{N}$  values (Fig. 5). Symbiotic associations with microorganisms that have access to N sources not available for plants can affect plant N isotope composition; however, all plant species investigated were non- $\text{N}_2$  fixing species. Further, level and type of mycorrhizal association have been shown to affect plant  $\delta^{15}\text{N}$  (Michelsen et al. 1998). In this study mycorrhizal colonisation by dark septate and arbuscular mycorrhizal fungi did not change with increasing altitude and were quite variable, although others have found fungal colonisation of roots to decrease with altitude in alpine regions (Haselwandter and Read 1980).

Competition and nutrient limitations may also influence the  $\delta^{15}\text{N}$  of plant material. On Mt. Schrankogel plant–plant competition was visibly less at the open nival vegetation (higher altitude) than at the alpine grassland site (lower altitude). This is further corroborated since the effect of neighbouring plants on each other changes with altitude from competition to facilitation in alpine communities (Brooker et al. 2005; Callaway et al. 2002; Choler et al. 2001;

**Fig. 6** Relationship between foliar  $\delta^{15}\text{N}$  of four alpine plant species and altitude at Mt. Schrankogel. Data points represent all individuals ( $n=15$ ) of four selected species, *Leucanthemopsis alpina*, *Luzula spicata*, *Poa laxa* and *Saxifraga bryoides*. These species were sampled from all five study plots on Mt. Schrankogel between 2,906 and 3,079 m. Results of a polynomial regression analysis (second order model) are given



Kikvidze et al. 2005). Limited N supply to plants – as may be the case in our lowest altitude plot – has reportedly resulted in no or only little N isotope fractionation (Evans et al. 1996; McKee et al. 2002; Nadelhoffer and Fry 1994). Consequently, more efficient uptake of N by plant roots would lead to less depleted foliar  $\delta^{15}\text{N}$  values which were not evident in this study, comparing the divergence between plant and soil  $\delta^{15}\text{N}$  with decreasing altitude.

Temperton et al. (2007) found that plant  $\delta^{15}\text{N}$  signatures in a biodiversity experiment decreased with increasing diversity of the plant assemblages. This suggested that changes in mineralization and or nitrification rates in more diverse versus less diverse plant assemblages could produce different  $\delta^{15}\text{N}$  signals in plants. We did not find a similar but an inverse relationship between plant  $\delta^{15}\text{N}$  and plant diversity, however, plant and soil  $\delta^{15}\text{N}$  values were also likely controlled by soil microbial processes that result in  $^{15}\text{N}$  depletion of soil inorganic N and consequently of plant N (Amundson et al. 2003).

It can therefore be speculated that the different ecosystems across the alpine–nival ecotone may differently respond to climate and atmospheric change. At the nival zone, where N limitation is not likely, an increase in atmospheric N deposition may not affect plant primary production. However, warmer temperatures may directly lead to increased plant growth and altered plant composition (Pauli et al. 2007) and may also increase rates of N mineralization. In contrast, at the alpine sites, where plant growth may be N limited (Callaway et al. 2002) due to stronger competition, it may be speculated that increased atmospheric deposition of N could markedly affect plant productivity and lead to changes in community structure.

In summary, we could show in this study that ecosystem nitrogen dynamics changed remarkably along a short altitudinal gradient (173 m) at the alpine–nival ecotone on Mt. Schrankogel. Our data from the field and from laboratory experiments suggest that temperature, soil age and development and plant competition all influence ecosystem N cycling, although to a varying extent at the different ecosystems.

**Acknowledgements** We are highly grateful to Alexandra Kaniak and Angelika Kaufmann for help in the field and Tina Bell and Ansgar Kahmen for comments on earlier versions of the manuscript. We further acknowledge the University of Vienna for travel funds to A. Richter and W. Wanek.

## References

- Adger N, Aggarwal P, Agrawala S, Alcamo J, Allali A, Anisimov O, Arnell N, Boko M, Canziani O, Carter T, Casassa G, Confalonieri U, Cruz RV and al. e (2007) IPCC 2007 climate change 2007: impacts, adaptation and vulnerability. Working Group II Contribution to the Intergovernmental Panel on Climate Change Fourth Assessment Report. Cambridge University Press. p 23
- Amato M, Ladd JN (1988) Assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soil. *Soil Biol Biochem* 20:107–114
- Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, Kendall C, Uebersax A, Brenner D, Baisden WT (2003) Global patterns of the isotopic composition of soil and plant nitrogen. *Global Biogeochemical Cycles* 17, Art. no. 1031
- Atkin OK, Cummins WR (1994) The effect of root temperature on the induction of nitrate reductase activities and nitrogen uptake rates in Arctic plant species. *Plant Soil* 159:187–197
- Barrett JE, Burke IC (2000) Potential nitrogen immobilization in grassland soils across a soil organic matter gradient. *Soil Biol Biochem* 32:1707–1716
- Berendse F, Lammerts EJ, Olf H (1998) Soil organic matter accumulation and its implications for nitrogen mineralization and plant species composition during succession in coastal dune slacks. *Plant Ecol* 137:71–78
- Bowman WD, Steltzer H, Rosenstiel TN, Cleveland CC, Meier CL (2004) Litter effects of two co-occurring alpine species on plant growth, microbial activity and immobilization of nitrogen. *Oikos* 104:336–344
- Brenner DL, Amundson R, Baisden WT, Kendall C, Harden J (2001) Soil N and  $^{15}\text{N}$  variation with time in a California annual grassland ecosystem. *Geochim Cosmochim Acta* 65:4171–4186
- Brooker R, Kikvidze Z, Pugnaire FI, Callaway RM, Choler P, Lortie CJ, Michalet R (2005) The importance of importance. *Oikos* 111:208–208
- Callaway RM, Brooker RW, Choler P, Kikvidze Z, Lortie CJ, Michalet R, Paolini L, Pugnaire FI, Newingham B, Aschehoug ET, Armas C, Kikvidze D, Cook BJ (2002) Positive interactions among alpine plants increase with stress. *Nature* 417:844–848
- Chapin FS, Shaver GR, Giblin AE, Nadelhoffer KJ, Laundre JA (1995) Responses of Arctic tundra to experimental and observed changes in climate. *Ecology* 76:694–711
- Choler P, Michalet R, Callaway RM (2001) Facilitation and competition on gradients in alpine plant communities. *Ecology* 82:3295–3308
- Cox JA, Whelan RJ (2000) Soil development of an artificial soil mix: nutrient dynamics, plant growth, and initial physical changes. *Aust J Soil Res* 38:465–477
- de Kovel CGF, Van Mierlo A, Wilms YJO, Berendse F (2000) Carbon and nitrogen in soil and vegetation at sites differing in successional age. *Plant Ecol* 149:43–50
- Dullinger S (1998) *Vegetation des Schrankogel, Stubai Alpen*. University of Vienna, Vienna
- Evans RD, Bloom AJ, Sukrapanna SS, Ehleringer JR (1996) Nitrogen isotope composition of tomato (*Lycopersicon esculentum* Mill. cv. T-5) grown under ammonium or nitrate nutrition. *Plant Cell Environ* 19:1317–1323

- Garnett TP, Smethurst PJ (1999) Ammonium and nitrate uptake by *Eucalyptus nitens*: effects of pH and temperature. *Plant Soil* 214:133–140
- Gottfried M, Pauli H, Grabherr G (1998) Prediction of vegetation patterns at the limits of plant life: a new view of the alpine–nival ecotone. *Arct Alp Res* 30:207–221
- Gottfried M, Pauli H, Reiter K, Grabherr G (1999) A fine-scaled predictive model for changes in species distribution patterns of high mountain plants induced by climate warming. *Divers Distrib* 5:241–251
- Gottfried M, Pauli H, Reiter K, Grabherr G (2002) Potential effects of climate change on alpine and nival plants in the Alps. In: Körner C, Spehn EM (eds) *Mountain biodiversity: a global assessment*. Parthenon Publishing, London, New York, pp 213–223
- Grabherr G, Gottfried M, Pauli H (1994) Climate effects on mountain plants. *Nature* 369:448–448
- Grogan P, Chapin FS (2000) Initial effects of experimental warming on above- and belowground components of net ecosystem CO<sub>2</sub> exchange in arctic tundra. *Oecologia* 125:512–520
- Handley LL, Raven JA (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant Cell Environ* 15:965–985
- Hartley AE, Neill C, Melillo JM, Crabtree R, Bowles FP (1999) Plant performance and soil nitrogen mineralization in response to simulated climate change in subarctic dwarf shrub heath. *Oikos* 86:331–343
- Haselwandter K, Read DJ (1980) Fungal associations of roots of dominant and sub-dominant plants in high-alpine vegetation systems with special reference to mycorrhiza. *Oecologia* 45:57–62
- Hobbie SE (1996) Temperature and plant species control over litter decomposition in Alaskan tundra. *Ecol Monogr* 66:503–522
- Högberg P (1997) Tansley review No 95 – <sup>15</sup>N natural abundance in soil–plant systems. *New Phytol* 137:179–203
- Jacot KA, Luscher A, Nosberger J, Hartwig UA (2000a) The relative contribution of symbiotic N<sub>2</sub> fixation and other nitrogen sources to grassland ecosystems along an altitudinal gradient in the Alps. *Plant Soil* 225:201–211
- Jacot KA, Luscher A, Nosberger J, Hartwig UA (2000b) Symbiotic N<sub>2</sub> fixation of various legume species along an altitudinal gradient in the Swiss Alps. *Soil Biol Biochem* 32:1043–1052
- Jonasson S, Havstrom M, Jensen M, Callaghan TV (1993) In-situ mineralization of nitrogen and phosphorus of Arctic soils after perturbations simulating climate change. *Oecologia* 95:179–186
- Jonasson S, Michelsen A, Schmidt IK, Nielsen EV (1999) Responses in microbes and plants to changed temperature, nutrient, and light regimes in the arctic. *Ecology* 80:1828–1843
- Jonasson S, Castro J, Michelsen A (2004) Litter, warming and plants affect respiration and allocation of soil microbial and plant C, N and P in arctic mesocosms. *Soil Biol Biochem* 36:1129–1139
- Kandeler E, Gerber H (1988) Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biol Fertil Soils* 6:68–72
- Khan SA, Mulvaney RL, Brooks PD (1998) Diffusion methods for automated nitrogen-15 analysis using acidified disks. *Soil Sci Soc Am J* 62:406–412
- Kikvidze Z, Pugnaire FI, Brooker RW, Choler P, Lortie CJ, Michalet R, Callaway RM (2005) Linking patterns and processes in alpine plant communities: a global study. *Ecology* 86:1395–1400
- Klanderud K, Birks HJB (2003) Recent increases in species richness and shifts in altitudinal distributions of Norwegian mountain plants. *Holocene* 13:1–6
- Kramer MG, Sollins P, Sletten RS, Swart PK (2003) N isotope fractionation and measures of organic matter alteration during decomposition. *Ecology* 84:2021–2025
- Kullman L (2002) Rapid recent range-margin rise of tree and shrub species in the Swedish Scandes. *J Ecol* 90:68–77
- McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective-measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytol* 115:495–501
- McKee KL, Feller IC, Popp M, Wanek W (2002) Mangrove isotopic ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) fractionation across a nitrogen vs. phosphorus limitation gradient. *Ecology* 83:1065–1075
- Metz B, Davidson OR, Bosch PR, Dave R, Meyer LA (2007) IPCC, 2007. *Climate change 2007: mitigation. Contribution of Working group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK, New York, p 35
- Michelsen A, Jonasson S, Sleep D, Havstrom M, Callaghan TV (1996) Shoot biomass,  $\delta^{13}\text{C}$ , nitrogen and chlorophyll responses of two arctic dwarf shrubs to in situ shading, nutrient application and warming simulating climatic change. *Oecologia* 105:1–12
- Michelsen A, Quarmby C, Sleep D, Jonasson S (1998) Vascular plant <sup>15</sup>N natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. *Oecologia* 115:406–418
- Moiseev PA, Shiyatov SG (2003) Vegetation dynamics at the tree line ecotone in the Ural Highlands, Russia. In: Nagy L, Grabherr G, Körner C, Thompson DBA (eds) *Alpine biodiversity in Europe*. Springer, Berlin Heidelberg New York, pp 423–435
- Morecroft MD, Marrs RH, Woodward FI (1992) Altitudinal and seasonal trends in soil nitrogen mineralization rate in the Scottish Highlands. *J Ecol* 80:49–56
- Munroe JS, Bockheim JG (2001) Soil development in low-arctic tundra of the northern Brooks Range, Alaska, USA. *Arct Antarct Alp Res* 33:78–87
- Nadelhoffer KJ, Fry B (1994) Nitrogen isotope studies in forest ecosystems. In: Lajtha K, Michener RH (eds) *Stable isotopes in ecology and environmental science*. Blackwell Scientific Publications, Oxford
- Nadelhoffer KJ, Giblin AE, Shaver GR, Laundre JA (1991) Effects of temperature and substrate quality on element mineralization in six Arctic soils. *Ecology* 72:242–253
- Patzelt J (1976) *Geologische Kartierung des Ötztalkristallins im Gebiet südlich der Amberger Hütte*. Diplomkartierung Universität Aachen. University of Aachen, Aachen
- Pauli H, Gottfried M, Grabherr G (1999) Vascular plant distribution patterns at the low-temperature limits of plant life – the alpine–nival ecotone of Mount Schrankogel (Tyrol, Austria). *Phytocoenologia* 29:297–325

- Pauli H, Gottfried M, Grabherr G (2001) High summits of the Alps in a changing climate. The oldest observation series on high mountain plant diversity in Europe. In: Walther G-R, Burga CA, Edwards PJ (eds) "Fingerprints" of climate change: adapted behaviour and shifting species ranges. Kluwer Academic Publisher, New York, pp 139–149
- Pauli H, Gottfried M, Reier K, Klettner C, Grabherr G (2007) Signals of range expansions and contractions of vascular plants in the high Alps: observations (1994–2004) at the GLORIA\*master site Schrankogel, Tyrol, Austria. *Glob Chang Biol* 13:147–156
- Peterjohn WT, Melillo JM, Steudler PA, Newkirk KM, Bowles FP, Aber JD (1994) Responses of trace gas fluxes and N availability to experimentally elevated soil temperatures. *Ecol Appl* 4:617–625
- Schinner F (1982) Soil microbial activities and litter decomposition related to altitude. *Plant Soil* 65:87–94
- Schmidt IK, Jonasson S, Shaver GR, Michelsen A, Nordin A (2002) Mineralization and distribution of nutrients in plants and microbes in four arctic ecosystems: responses to warming. *Plant Soil* 242:93–106
- Shaw MR, Harte J (2001) Response of nitrogen cycling to simulated climate change: differential responses along a subalpine ecotone. *Glob Chang Biol* 7:193–210
- Steltzer H, Bowman WD (1998) Differential influence of plant species on soil nitrogen transformations within moist meadow Alpine tundra. *Ecosystems* 1:464–474
- Stuiver M, Polach HA (1977) Reporting of  $^{14}\text{C}$  data – discussion. *Radiocarbon* 19:355–363
- Sveinbjornsson B, Davis J, Abadie W, Butler A (1995) Soil carbon and nitrogen mineralization at different elevations in the Chugach Mountains of South-Central Alaska, U.S. *A. Arct Alp Res* 27:29–37
- Temperton VM, Mwangi PN, Scherer-Lorenzen M, Schmid B, Buchmann N (2007) Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. *Oecologia* 151:190–205
- van Heerwaarden LM, Toet S, Aerts R (2003) Nitrogen and phosphorus resorption efficiency and proficiency in six sub-arctic bog species after 4 years of nitrogen fertilization. *J Ecol* 91:1060–1070
- Vierheilig H, Schweiger P, Brundrett M (2005) An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiol Plant* 125:393–404
- Virtanen R (2003) The high mountain vegetation of the Scandes. In: Nagy L, Grabherr G, Körner C, Thompson DBA (eds) *Alpine biodiversity in Europe*. Springer, Berlin, pp 31–38
- Volder A, Bliss LC, Lambers H (2000) The influence of temperature and nitrogen source on growth and nitrogen uptake of two polar-desert species, *Saxifraga caespitosa* and *Cerastium alpinum*. *Plant Soil* 227:139–148
- Walther GR, Beissner S, Burga CA (2005) Trends in the upward shift of alpine plants. *J Veg Sci* 16:541–548
- Watson RT, Zinyowera MC, Moss RH, Dokken DJ (1997) IPCC special report. The regional impacts of climate change: an assessment of vulnerability. Cambridge University Press, Cambridge, U.K., p 527