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List of Abbreviations

CFE — Chloroform Fumigation Extraction

CO_2 — Carbon dioxide

DNRA — Dissimilatory Nitrate Reduction to Ammonium

DON — Dissolved Organic Nitrogen

HMW-ON — High-molecular weight organic nitrogen

lmer — linear mixed effects model

LMW-ON — Low-molecular weight organic nitrogen

MAT — Mean Annual Temperature

MAP — Mean Annual Precipitation

NH_4^+ — Ammonium

N_{mic} — Microbial Nitrogen

NO_3^- — Nitrate

PCA — Principal Components Analysis

SOM — Soil Organic Matter

TDN — Total Dissolved Nitrogen

1. Abstract

Climate change and biogeochemical cycles are strongly interlinked. The anthropogenically induced rise of atmospheric CO_2 connects the carbon with the nitrogen cycle as climate warming may accelerate microbial decomposition processes of soil organic matter. This affects biogeochemical processes in all ecosystems on a global scale. Especially high altitude/latitude ecosystems like montane forests are disproportionately affected, as they are particularly sensitive to increasing temperatures. To consolidate the existing knowledge about the responses of terrestrial nitrogen cycling processes to climate change, this master thesis aimed to study the effects of long-term soil warming on the nitrogen cycle in montane forest soils.

For this, natural ^{15}N-isotopic natural abundances (expressed as $\delta^{15}N$ values) of soil nitrogen pools were measured in a long-term soil warming experiment in Achenkirch, Austria. Besides a significant seasonal effect on $\delta^{15}N$ signatures of all pools, I found a warming effect on the isotopic signatures of root nitrogen and the soil NH_4^+ pool, i.e. a ^{15}N enrichment in these pools. As roots can only be considered an integrator of inorganic soil nitrogen processes, the results suggest elevated isotope fractionation in the NH_4^+ pool through increased nitrification and therefore an opening of the nitrogen cycle in warmed plots with greater soil nitrogen losses.

Natural ^{15}N abundance studies hold great potential for evaluating the status quo of the nitrogen cycle in terrestrial ecosystems and to monitor *in situ* responses to climate change with minimal invasion. To fully grasp potential changes in the biogeochemical nitrogen cycle triggered by climate change, more ecosystems need to be studied and synthesized in global meta-analyses.

Climate Change, ^{15}N natural abundance, long time warming, N-cycle, nitrification.

Abstrakt in deutscher Fassung

Klimawandel und biogeochemische Kreisläufe sind eng miteinander verknüpft. Der anthropogen bedingte Anstieg des atmosphärischen CO_2 verbindet den Kohlenstoff- mit dem Stickstoffkreislauf, da eine Klimaerwärmung mikrobielle Zersetzungsprozesse organischer Bodensubstanz beschleunigen kann. Dies wirkt sich auf biogeochemische Prozesse in allen Ökosystemen aus. Besonders hohe und in hohen Breitengraden gelegene Ökosysteme wie beispielsweise Bergwälder sind überproportional betroffen, da sie besonders empfindlich auf steigende Temperaturen reagieren. Um das vorhandene Wissen über die Reaktionen des terrestrischen Stickstoffkreislaufs und dessen Prozesse auf den Klimawandel zu festigen, zielte diese Masterarbeit darauf ab, die Auswirkungen von Klimaerwärmung auf den Stickstoffkreislauf in montanen Waldböden zu untersuchen.

Dazu wurden in einem Langzeitversuch zur Bodenerwärmung in Achenkirch, Österreich, natürliche ^{15}N-Isotopenhäufigkeiten (ausgedrückt als $\delta^{15}N$-Werte) von Bodenstickstoffpools gemessen. Neben einem signifikanten saisonalen Effekt auf die $\delta^{15}N$-Signaturen aller Pools konnte ich einen Erwärmungseffekt auf die Isotopensignaturen des Wurzelstickstoffs und des Boden-NH_4^+-Pools, d.h. eine ^{15}N-Anreicherung in diesen Pools, finden. Da Wurzeln nur als Integrator anorganischer Bodenstickstoffprozesse interpretiert werden können, deuten die Ergebnisse auf erhöhte Isotopenfraktionierung im NH_4^+-Pool durch gesteigerte Nitrifizierung und damit auf eine Öffnung des Stickstoffkreislaufs in erwärmten Plots mit höheren Bodenstickstoffverlusten hin.

Studien zur natürlichen ^{15}N-Häufigkeit bergen ein großes Potenzial für die Bewertung des Status quo des Stickstoffkreislaufs in terrestrischen Ökosystemen und für die Überwachung von *in situ* Reaktionen auf den Klimawandel mit minimaler Invasion. Um potenzielle Veränderungen im biogeochemischen Stickstoffkreislauf, die durch den Klimawandel ausgelöst werden, vollständig zu erfassen, müssen mehr Ökosysteme untersucht und in globalen Metaanalysen zusammengefasst werden.

2. General Introduction

2.1. Climate Change and Biogeochemical Cycles

Climate is changing. Mankind has left the relatively stable Holocene for a new geologic era, the so called Anthropocene (Crutzen, 2006). Human activities influence global biogeochemical cycles which are inherently connected to each other (Gruber & Galloway, 2008). The most prominently affected biogeochemical cycle is the carbon cycle, which has been altered through fossil fuel burning and land use change over decades, leading to a strong increase in the concentration of atmospheric enrichment of carbon dioxide (CO_2). This anthropogenically affected carbon cycle is strongly connected and interlinked with other biogeochemical cycles, especially the nitrogen cycle.

Besides phosphorus, nitrogen is one of the most important nutrient elements in nature and it is therefore of uttermost importance to study its behavior in modern science and in relation to global change. Nitrogen is essential for life and the growth of all organisms as it is a constituent of proteins, amino acids, co-factors, RNA and DNA, secondary compounds, and microbial cell walls in the form of chitin and peptidoglycan. It can form complex organic molecules, which can be broken down to inorganic forms by mineralization processes. However, the biggest pool of nitrogen on earth is the atmosphere with nitrogen present as dinitrogen (N_2). This high diversity of nitrogen forms reflects the multitude of transformation steps in this element cycle, but it also shows it's complexity as Galloway et al. (2004) state:

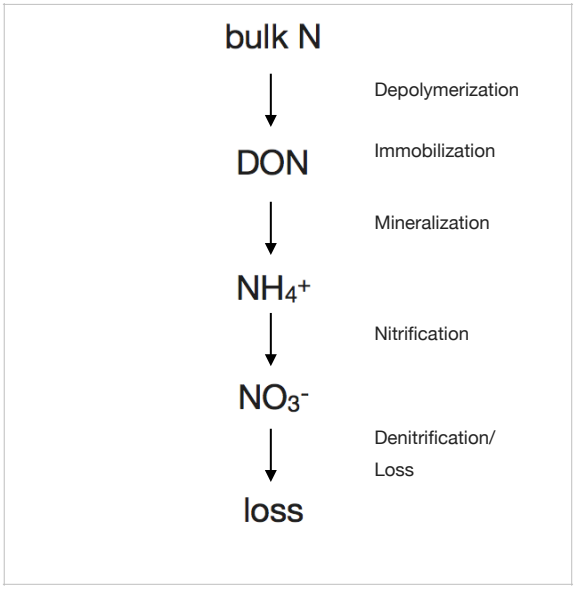
> *"With seven oxidation states, numerous mechanisms for interspecies conversion, and a variety of environmental transport/storage processes, nitrogen has arguably the most complex cycle of all the major elements."*

The carbon and nitrogen cycle are strongly linked through similar actors like microbial organisms in the soil or plants performing primary production (Nave et al., 2009; Hopkins et al., 1998), which makes it possible that these actors become competitors for nitrogen (Rütting et al., 2010). Also, nitrogen availability has been suggested to exert an indirect control on „forest soil C dynamics through effects on organic matter decomposition" (Garten et al., 2007). The human impact on these biogeochemical cycles is undeniable: It is estimated that anthropogenic reactive nitrogen inputs to soils enabled the sequestration of 11.2 Pg carbon, and that the CO_2 fertilization effect is estimated to have led to an additional carbon

storage of 1.2 Pg by promoting plant growth (Zaehle, 2013). The biogeochemical cycles are accelerating and climate change are both trigger and consequence of this. Questions like how does the availability of nitrogen affect the capacity of the Earth's biosphere to continue absorbing CO_2 from the atmosphere are becoming more and more pressing.

One of the most important climatic impacts of the altered carbon cycle on the nitrogen cycle comes from climate warming caused by an increasing atmospheric concentration of potent greenhouse gases. A rising CO_2 concentration in the atmosphere is directly physically linked to increasing global temperatures (Jouzel et al., 2007; Shakun et al., 2012). Global warming also causes soil temperatures to increase, but if winter soils become snow cover-free, soils will cool down in winter due to the lack of the insolating snow cover. This is important, as changes in global temperatures are expected to strongly impact transformation steps in biogeochemical cycles like the nitrogen cycle. The impacts of global warming can be manifold: Elevated temperatures positively correlate with soil nitrogen availability (Dawes et al., 2017; Melillo et al., 2011), generally indicating a change in nitrogen input and output rates (Olson, 1958) and an opening of the nitrogen cycle. A change in nitrogen availability can have an impact on the nitrogen use efficiency (NUE) of microbes, altering the decomposing process (Mooshammer et al., 2014). While an altered enzyme activity in warmed soils is still a matter of debate, as several studies showed contradicting results (Schindlbacher et al., 2015; Bell et al., 2010; Cookson et al., 2007; Feng et al., 2007), net N mineralization and net nitrification (Melillo et al., 2002; Bai et al., 2013) as well as fine-root biomass (Wang et al., 2021) increase significantly. However, it needs to be considered, that warming effects on N cycling can be different in extent and direction in different ecosystems, as the interaction of temperature with other abiotic factors can modulate the response (Bai et al., 2013).

Ecosystem sciences are getting closer to fully understanding the black box of terrestrial nitrogen cycling by unraveling the single processes and feedbacks. Dissecting the ecosystem nitrogen flux into single processes makes it possible to approach the full elucidation of the nitrogen cycle and of its component processes for the first time. This is important to note, because the nitrogen cycle is not balanced as the six prevalent nitrogen processes are associated with nitrogen fluxes of vastly different magnitude (Kuypers et al., 2018), though many authors ignored one of the most dominant processes, the depolymerization of complex organic nitrogen molecules to labile assimilable ones. These six (seven) classical processes are: nitrogen fixation, assimilation, mineralization (ammonification), nitrification, denitrification, anammox (and depolymerization). We here adopted a practical and



Depolymerization	Breakdown of a polymer containing nitrogen into a monomer.
Immobilization	Uptake of nitrogen by microorganisms.
Mineralization	Conversion of nitrogen by microorganisms and release as ammonium.
Nitrification	Conversion of ammonium to nitrate by chemoautotrophic bacteria.
Denitrification	Conversion of nitrate into pure nitrogen. Loss of nitrogen through gaseous form.

Fig. 1: Schematic representation of crucial nitrogen pools and conversion processes. Definitions following Hopkins et al. (1998).

holistic perspective on the soil level: High-molecular weight organic nitrogen (HMW-ON) forms that dominate the soil nitrogen reservoir are first depolymerized into small oligomers and monomers by extracellular enzymes secreted by soil microbes and plants, followed by the uptake of these low-molecular weight organic nitrogen (LMW-ON) forms by microbes (immobilization). Bacteria use those as energy and nitrogen source; the excess of nitrogen being mineralized to ammonium, which is excreted into the environment. Ammonium is either assimilated by microbes or plants or used by chemoautotrophic bacteria and archaea for energy production and converted into nitrate via nitrite (nitrification). Nitrate is highly susceptible to loss from soils, as it can be denitrified causing gaseous losses in the form of NO, N_2O and N_2, or is simply lost via leaching (Fig. 1) if it is not assimilated by organisms. It can also be reconverted to ammonium by bacteria performing dissimilatory nitrate reduction to ammonium (DNRA).

2.2. Introduction to Stable Isotopes in Ecology

The word isotope derives from the two greek words *iso* and *topos*, translating into „equal place“. In the applied physical context this means that isotopes form a group of nuclides with the same number of protons and electrons, but they differ in their number of neutrons and therefore in their mass — but still hold the same place in the periodic system of elements. Isotopes behave chemically similar, as they have the same electron number and configuration, but their mass and the physical behavior of different isotopes deviates quan-

titatively (Sulzman, 2007). In a mass spectrometer, isotopically heavier molecules are less deflected by electromagnetic force than their isotopically lighter counterparts and have therefore different flight paths (Aston, 1919). But it's not only the flight path that differs between heavy and light isotopes: heavier isotopes also form bonds more slowly, which are harder to break as more energy is needed (Bigeleisen, 1965), causing discrimination against heavier isotopes during bond formation and breakage, termed isotope fractionation.

Moreover, we distinguish between unstable and stable isotopes. While unstable isotopes emit radioactive radiation during radioactive decay to form more stable isotopes, stable isotopes do not decay but remain stable and are the product of the decay of radioactive isotopes (Kendall & Caldwell, 1998). They remain stable for measurable time periods (billions of years). Elements with stable isotopes states like oxygen, hydrogen, carbon, sulfur and nitrogen consitute the light elements forming the largest fraction of living and dead organic matter and are therefore of great importance in ecological studies of element cycling as „[i]sotopes are something like a mysterious hidden language written everywhere in the common chemicals and compounds circulating in the biosphere" (Fry, 2006). Isotopes can be used as tracers — at natural abundance given that isotope fractionation differs between key processes or that different sources of utilized nitrogen deviate in isotope composition, or by following the fate of tracers enriched with the rarer, heavier isotope.

2.2.1. Quantification of Stable Isotopes

To quantify stable isotopes, their abundance in different pools and isotope fractionation through different physical and biochemical processes are measured and calculated. While isotopic changes happen on a very small scale, such changes can be highly process- and context-specific and therefore convey information on the underlying processes and changes of them. For isotope calculations a range of different expressions and equations are applied. Measuring the natural **abundance** of a heavy isotope relative to its lighter counterpart leads to the expression of these isotopic abundances in the so called delta notation. This is based on isotope ratios, R, where the ratio of an accepted international standard ($R_{standard}$) is compared to the ratio of the measured sample (R_{sample}):

$$\delta^{15}N\ (‰) = ((R_{sample} / R_{standard}) - 1) \times 1000 \text{ where}$$

$$R = {}^{15}N{:}{}^{14}N$$

The δ value is expressed in ‰ (per mille or per thousands), which refers to the deviation of the ^{15}N:^{14}N ratio (R) between a sample and an international standard, to visualize the small differences in the isotope ratios between samples (Högberg, 1997). A sample with a δ value of 10‰ is 1% higher in its ^{15}N:^{14}N ratio compared to the standard. The standard always has a δ value of 0‰; in the case of nitrogen, this standard is defined by dinitrogen (N_2) in the atmosphere, which has an isotope ratio of 0.0036765 and the atom ^{15}N percentage (at%^{15}N) is 0.36630% compared to that of ^{14}N of 99.6337% (Junk & Svec, 1958; Mariotti et al., 1981). Applying this logic, a positive δ value refers to an enrichment of the sample with ^{15}N relative to atmospheric N_2, whereas a negative δ value suggests the opposite (Dawson & Siegwolf, 2007).

Besides isotopic abundances, the **fractionation** factor (α) is an important value to categorize element cycling processes. Isotopic fractionation happens during physical and biochemical reactions, because more energy is needed to transform molecules with heavier isotopes (Hopkins et al., 1998; Robinson, 2001). Högberg (1997) differentiates between two types of isotope fractionation: equilibrium isotope effects (1) and kinetic isotope effects (2):

$$(1)\ \alpha = \delta_A/\delta_B$$
$$(2)\ \alpha = k_L/k_H,$$

where $\delta_{A/B}$ refers to the δ values of the two different measured pools and $k_{L/H}$ to the process rates (kinetic constants) of the lighter (L) and heavier (H) isotopic molecules. The fractionation factor α has no unit, however it transforms the reaction rate of isotopes to a more manageable value: For instance, a „1% faster reaction of k_L versus k_H translates to an α value of 1.01“ (Fry, 2006). This fractionation factor can be further processed to an equation, leading to an expression for isotope **fractionation** or isotope **discrimination** (Δ or ε):

$$\varepsilon = (\alpha - 1) \times 1000 \text{ or}$$
$$\Delta = \delta_s - \delta_p = ((\delta_s - \delta_p)/(1+ \delta_p/1000))$$

Isotope fractionation is also expressed in ‰ and refers to the change of the δ value from the residual substrate to the δ value of the product (Högberg, 1997). These arrangements of isotope values and of isotope fractionation factors in stable isotope applications make

isotope measurements the perfect tool for ecologists to understand biogeochemical cycles and ecological/biological processes.

2.2.2. Stable Nitrogen Isotope Approaches

Nitrogen as an element containing two stable isotopes can be quantified isotopically and isotope values can be calculated as explained in the previous chapter. As deepening the understanding of the nitrogen cycle is crucial in the view of climate and land use change, nitrogen holds an important role in the application of stable isotope studies to ecological systems. Natural ^{15}N abundance methods cover a broad application spectrum and have also been used to track nitrate pollution, to study food-web dynamics or plant nitrogen acquisition strategies in different successional stages of ecosystems (Wang et al., 2010; Zhang et al., 2018). The high complexity and diversity of nitrogen transformation processes and nitrogen pools makes ^{15}N isotope applications a powerful tool to study the nitrogen cycle and to follow possible changes thereof triggered by land use change or climate warming.

One possibility to identify, specify and determine these fluxes is by quantifying nitrogen isotopes — ^{15}N and ^{14}N — of the different nitrogen pools. Every conversion step of nitrogen discriminates more or less strongly against the heavier nitrogen isotope ^{15}N leading to isotope fractionation — a difference in the ratio between heavy and light nitrogen. Measuring the proportion of the rarer ^{15}N in the pools of the nitrogen cycle makes it possible to calculate the isotope fractionation in each conversion step as well as the fraction of a specific substrate being transformed to the next product. Isotope fractionation generates a specific isotopic signature or fingerprint in the respective substrates or products (Craine et al., 2009). There are many possible factors influencing this isotopic fingerprint on the multitude of soil nitrogen pools and the level of isotope fractionation leading to a distinctive pattern, which can then be interpreted in the light of the underlying key processes.
Besides soil carbon and nitrogen availability, microbial processing of nitrogen (depending on labile C and N concentrations), precipitation and texture (clay), ^{15}N-isotope fractionation strongly depends on environmental temperature (Craine et al., 2014; Högberg, 1997). Given this environmental sensitivity of nitrogen isotope fractionation and nitrogen flux partitioning in the nitrogen cycle, stable isotope approaches hold a big potential in quantifying differences and revealing changes in the nitrogen cycle.

The general process of ^{15}N enrichment of soils and the differential ^{15}N enrichment or ^{15}N depletion of specific nitrogen pools is based on the nature and characteristics of the underlying nitrogen cycle processes (transformation steps), i.e. their isotope fractionation and the fraction of substrate consumption. Early and later steps of the nitrogen cycle are important in this context as they strongly differ in their potential isotope fractionation. Especially gaseous losses through denitrification (NO, N_2O and N_2) and leaching of nitrate are of importance as they can lead to an opening of the nitrogen cycle and an accumulation of ^{15}N of residual nitrogen in the soil (Craine et al., 2009). These inorganic nitrogen cycle processes are driven by nitrifiers (followed by hydrological export) and denitrifiers. Both groups of microorganisms exert a strong isotope effect causing their product to become ^{15}N depleted and their residual substrate to become successively ^{15}N enriched. Soil microorganisms act as „valves“ between the different nitrogen pools and control the flow of nitrogen and their stable isotopes, ^{15}N and ^{14}N, and define the isotopic composition of their initial substrates (IS), the residual substrates (RS) and the cumulative products (CP) of the interconnected nitrogen pools according to the Rayleigh isotope fractionation model (Xu et al., 2021; Mariotti et al. 1981). Understanding this model is of crucial importance as it is the physical basis of the process-based isotope fractionation of nitrogen pools in the nitrogen cycle, causing the mentioned isotopic fingerprints in the different soil nitrogen pools. It shows „an exponential relation that describes the partitioning of isotopes between two reservoirs as one reservoir decreases in size“ (Kendall & Caldwell 1998). The IS is defined isotopically by the previous process injecting fresh substrate into the IS pool. During the consumption of this IS, where the fraction of substrate consumed increases, the RS becomes exponentially ^{15}N enriched while the CP that is formed is ^{15}N depleted. When all substrate has been consumed by this process the CP is isotopically identical to the IS (Fig. 2). In the next transformation process which uses the CP of the former process as IS, the former CP is redefined as IS and the calculations can begin again. According to this logic a

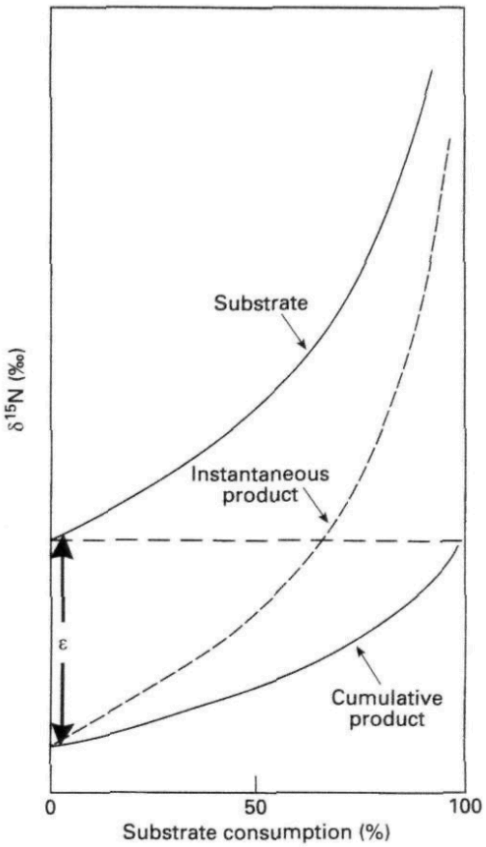


Fig. 2: Rayleigh fractionation in a closed system (Högberg, 1997).

pool „can become isotopically heavier than the organic N from which it is derived“ from (Högberg, 1997).

Enzymatic Isotope Fractionation

Different enzymes characterize each transformation step. While catalyzing the enzyme-mediated processes, enzymes discriminate more or less strongly against isotopically heavy molecules, which leads to a different ^{15}N:^{14}N ratio in individual nitrogen pools and to the development of individual isotopic fingerprints on each nitrogen pool. It is important to note here, that physical processes such as diffusion in water, sorption or uptake processes without biochemical transformation (bond formation or breakage) cause no or only very little isotope fractionation. When isotopically distinct nitrogen pools mix the resulting ^{15}N in the mixture represents the mass-weighted average of the ^{15}N of each component (Robinson, 2001).

Handley & Raven (1992) summarized from the available literature that enzymatic reactions like nitrogen fixation, transamination, N_2O reduction, denitrification, deamination, the assimilation of nitrate- (NO_3^-), nitrite- (NO_2^-) and ammonium- (NH_4^+), as well as amino acid synthetases and dehydrogenases all differently discriminate against ^{15}N in laboratory experiments. Only recently, dissimilatory nitrate reduction to ammonium (DNRA) has been discovered to contribute to isotope fractionation with values ranging from 10.8 ‰ to 34.8 ‰ (Asamoto et al., 2021). However, the evidence about isotope fractionation by enzymes is conflicting, due to a strong variability in the diversity of performing microorganisms and differences in their isotope fractionation (Denk et al., 2017). Table 1 shows the isotope fractionation factors of the most important nitrogen transformation processes according to different literature sources. It’s important to note that processes like nitrification, denitrification and DNRA, which are located at the end of the nitrogen cycle, discriminate more against ^{15}N than processes which operate at the beginning of the nitrogen cycle, like nitrogen fixation (Craine et al., 2009). Also, as nitrification will become an important role in the following study, it is important to state at this point that the average isotope fractionation value of 29.6 ‰ will be used for nitrification (ammonium oxidation to nitrite) according to Denk et al. (2017), as the oxidation from ammonium (NH_4^+) to nitrite (NO_2^-) represents the rate-determining step in this process.

Table 1: Isotope fractionation factors (ε in ‰) in the nitrogen cycle according to different sources. For nitrification Denk et al. (2017) give two values for each oxidation step. We here represent the values of $\varepsilon_{S/P}$, i.e. positive values of isotope fractionation indicate that the products are ^{15}N depleted.

Process	Denk et al. (2017)	Dawson & Siegwolf (2007)	Handley & Raven (1992)	Högberg (1997)
N_2 fixation	0.6 to 2.5	-3.7 to 3.9	9	-20 to 20
Mineralization (Ammonification)	1.74	-0.8 to 5.0	20	0
Nitrification	NH_4^+ to NO_2^-: 29.6 ± 4.9 NO_2^- to NO_3^-: 13.0 ± 1.5	5.4 to 34.7	9	15 to 35
Denitrification	NO_3^- to NO_2^-: 31.4 ± 11.8 NO_2^- to NO: 21.7 ± 6.7 NO to N_2O: -14.0 ± 1.6	17.3 to 40.0	33	0 to 33
Ammonia volatilization		25 to 35	-	29
Ammonium assimilation	9.4 ± 6.6	1.1 to 14.8	8 (plants)	0 to 20
Nitrate assimilation	19.2 to 0	1.1 to 4.9	20 (plants)	0 to 20
N_2O production: Nitrification	-	34.9 to 68.4	-	-
N_2O reduction	-	27 to 39	-	-

Environmental Influences on Isotope Fractionation

Several factors have been identified, which can influence the fractionation of nitrogen isotopes and the isotopic signatures of soils in general: Climate (temperature, precipitation and soil water availability), microbial community structure, the availability of nitrogen and other nutrients (carbon, phosphorus), soil depth and soil texture.

Temperature is an abiotic and physical factor, which influences all physical and biochemical processes, and therefore is the most crucial for modulating isotope fractionation on the enzymatic, organismic and ecosystem level. While the temperature effect on equilibrium isotope effects is large and decreases with increasing temperature, the temperature effect on kinetic isotope effects is much smaller to negligible (Kendall & McDonnell, 1998). Just like for other chemical bonds with heavy and light isotopes, more energy is needed to break these bonds when ^{15}N is involved (Robinson, 2001). Hence, temperature affects the

nitrogen cycle and isotope fractionation of its component processes on the level of molecular bonds, as enzymatic activity increases with increasing temperature and more energy for breaking these bonds is available. Isotope fractionation of most processes have been measured via *in vitro* enzymatic activity approaches or in pure microbial isolates, but rarely in complex soil microbial communities. Saad and Conrad (1993) for example observed the optimum temperature for the two processes nitrification and denitrification to be around 25-30 °C, indicating a connection between temperature and isotope fractionation at the ecosystem level, i.e. through changing the flux balance between different nitrogen cycle processes, thus affecting nitrogen cycling. Therefore, if climate change brings soil temperature closer to this optimum, nitrification and denitrification processes will increase (Bai et al., 2013), and the likelihood of soil nitrogen losses increases. Though other studies showed that temperature effects on isotope fractionation by enzymes are low; in soils with increasing temperatures enzymatic activity was shown to decrease (Maggi & Riley, 2015) or slightly increase isotope fractionation during biological denitrification (Wang et al., 2018). On the other hand, chemodenitrification, a non-biological process, showed a stronger increase in isotope fractionation from 5 to 25 °C, followed by a decrease towards 35 °C (Chen et al., 2021). Temperature effects on isotope are therefore complex to understand, variable within and across system levels and are partially idiosyncratic. On the ecosystem level the products of nitrifiers and denitrifiers are usually ^{15}N depleted inorganic or gaseous nitrogen forms, which are easily lost from an ecosystem, causing a ^{15}N enrichment of nitrogen remaining in the ecosystem. An increase in temperature could result in accelerated decomposition leading to an increase in mineralization and possibly nitrification, which would open the nitrogen cycle through increased losses (Philben et al., 2018). The same effect can be expected if soil nitrogen availability increases beyond a certain threshold, above which soil nitrogen losses increase. When the nitrogen cycle of a system opens, nitrogen losses can occur through gaseous or hydrological pathways. The type of these losses differ in their ^{15}N composition through different isotope fractionation factors and therefore affect the isotopic status of the respective nitrogen pools in soils (Koba et al., 2012). While gaseous losses, which emerge through the strongly isotope fractionating process of denitrification, heavily enrich soils with ^{15}N, losses through leaching is a physical process with no isotope fractionation. However, nitrification exerts a strong isotope effect causing nitrate to be ^{15}N depleted, which, when lost, also causes ecosystem ^{15}N enrichment. In a global meta-analysis of *in situ* studies on soil and plant $\delta^{15}N$ Amundson et al. (2003) demonstrated that with increasing mean annual temperature (MAT) soil $\delta^{15}N$ increases due to ^{15}N fractionating loss pathways. The same was demonstrated in a later meta-analysis

by Craine et al. (2014).

In the same meta-analyses by Amundson et al. (2003) and Craine et al. (2014) the role of mean annual **precipitation** (MAP) in terms of soil nitrogen isotope soil composition was highlighted. A decrease in precipitation was related to increasing ^{15}N enrichment in soils. Mean annual temperature is strongly connected to MAP as both factors play an important role in controlling soil nitrogen dynamics. They concluded that “hotter and drier ecosystems tend to lose a greater proportion of their N through gaseous pathways” (Craine et al., 2014), causing greater ^{15}N enrichment in these soils compared to cold-humid regions with a more closed nitrogen cycle.

Differences in discrimination factors for nitrogen transformation processes are also linked to diversity of the microbes mediating these processes (Denk et al., 2017). Differences in the **microbial community composition** of soils in different ecosystems can therefore be further modulators changing isotope fractionation pathways. However, it has been shown that the factor temperature did not strongly affect soil microbial community composition indicating a certain resistance of soil microbial communities (Ballhausen et al., 2020). Instead, thermal adaptations of soil microbial communities are widely observed and seem to be the rule rather the exception (Bradford, 2013; Schindlbacher, 2015; Luo et al., 2001).

Generally, a crucial parameter controlling nitrogen fluxes through different transformation pathways is substrate availability. **Nitrogen availability** is therefore key driver of soil nitrogen transformation processes and their related isotope fractionation. An excess of nitrogen in ecosystems can result in increased leaching or denitrification losses (Choi et al., 2020), leading to differences in the ^{15}N:^{14}N ratio of ecosystem nitrogen pools. However, several authors also stressed the leaching of DON (Neff et al., 2003), particularly from old grown forest soils (Hedin et al., 1995), meaning that not only nitrate is amenable to leaching losses. When nitrogen is less available, it is cycled more conservatively and incorporated into biomass as organic nitrogen (Craine et al., 2009). Moreover, soil and agricultural management such as fertilization (manure or synthetic fertilizer, mowing, grazing etc.) can strongly affect isotope dynamics; it has been shown that intensively grazed mountain pastures were ^{15}N enriched by 3.5‰ in comparison to less used pastures (Zech et al., 2011) or that amendment of organic instead of synthetic fertilizer cause plant and soil ^{15}N enrichment in managed grasslands in a rate-dependent way (Watzka et al., 2006). The influence of nitrogen availability on nitrogen isotope fractionation is, however, a complex one, given the influence of other nutrients such as phosphorus and potassium and of labile carbon on the ecosystem nitrogen cycle. Craine et al. (2014) observed a decrease in soil $\delta^{15}N$ at higher soil carbon contents and higher soil C:N ratios. Low soil carbon contents, howe-

ver, are found in dryer and hotter climates, biasing a direct effect of soil carbon on soil $\delta^{15}N$, given the co-variation of soil carbon, MAT and MAP.

Finally, soil **depth** and **texture** can strongly influence the $\delta^{15}N$ value. Many authors found a significant accumulation of heavy nitrogen in deeper soil layers (Delwiche & Steyn, 1970; Karamanos & Rennie, 1978), caused by (i) isotope fractionation during nitrogen transfer by mycorrhizal fungi to host plants, leading to ^{15}N depleted plant litter on topsoils and ^{15}N enriched nitrogen of fungal origin at greater soil depth, (ii) preferential preservation of ^{15}N enriched compounds during decomposition and stabilization, and (iii) cumulative nitrogen loss during nitrification and denitrification, causing large ^{15}N enrichments with depth (Hobbie & Ouimette, 2009). Also, the soil clay content is discussed to have a measurable impact on soil $\delta^{15}N$ (Craine et al., 2014). This is most likely explained by the fact that microbially-derived organic matter (microbial necromass, which is ^{15}N enriched relative to plant organic matter) is strongly sorbed by clay surfaces and thereby stabilized. The more microbially altered and recycled soil organic matter becomes, the larger the ^{15}N enrichment is of microbial necromass. Moreover, a reduction in clay particle size usually accompanies greater microbial degradation of organic matter (Hopkins et al., 1998).

It has been recently stated that soil $\delta^{15}N$ is the better integrator of ecosystem nitrogen cycling compared to plant $\delta^{15}N$ (Liao et al., 2021). However, others demonstrated that plant $\delta^{15}N$ is the better integrator of soil ecosystem nitrogen cycling and losses than soil $\delta^{15}N$ in the short and medium term as plant $\delta^{15}N$ respond faster to changes in agricultural management or other treatments compared to soils (Watzka et al., 2006).

Substrate ^{15}N enrichment and ^{15}N depletion of products of a process also depend on the substrate supply:demand ratio, which determines whether a system operates in an open system mode or in a closed system mode. The difference between these two types of system modes is the supply: While in closed systems there is no or little influx of substrate, open systems are characterized by a constant nitrogen input and loss (Zech et al., 2011). In other words „[i]n an open system, the supply of reactant is infinite; in a closed system, it is finite“ (Kendall & Caldwell, 1998). This has important repercussions on isotope fractionation patterns and of how to calculate the isotope composition of RS and CP in a process. In open system reactions a product is formed from a source (input, substrate) continuously, and both product and residual substrate exit the system or site of reaction. One can calculate the isotopic compositions of residual substrate (RS) and cumulative product (CP), depending on the fraction f of substrate converted to product, the isotope fractionation of the process (Δ) and isotopic composition of initial substrates (δ_{input}), using the following equations:

$$\delta_{RS} = \delta_{input} + \Delta * f$$

$$\delta_{CP} = \delta_{input} - \Delta * (1 - f)$$

In a closed system reaction, where a fraction of substrate is reacted to a product, the following applies (Rayleigh distillation equations):

$$\delta_{RS} = \delta_{input} - \Delta * ln(1 - f)$$

$$\delta_{CP} = \delta_{input} + \Delta * \frac{(1 - f)}{f} * ln(1 - f)$$

High mountain ecosystems have been shown to undergo significantly great climate warming compared to their lowland counterparts. Mountain ecosystems are believed to conservatively cycle nitrogen, with little losses indicative for a closed nitrogen cycle. Global warming is expected to accelerate nitrogen cycling in these systems and thereby open the nitrogen cycle leading to increased losses (Dawes et al., 2017). In the case of the Achenkirch warming experiment, manipulation of the factor temperature by +4 °C in a mixed spruce-beech forest mimics an extreme scenario of global warming. We hypothesize that soil warming in Achenkirch will accelerate soil nitrogen cycling and cause larger nitrogen losses, which will be reflected in an altered isotopic composition of soil nitrogen pools, i.e. becoming ^{15}N enriched. It is important to understand changes of the nitrogen cycle early in order to protect ecosystems under climate change and conserve crucial ecosystem services.

3. Manuscript

Long-term soil warming alters the nitrogen flux partitioning and causes increased nitrogen losses, as indicated by natural ^{15}N abundance measurements in fine roots and soil nitrogen pools in a montane forest.

3.1.Introduction

Climate change is expected to have a lasting impact on global ecosystems. Biogeochemical cycles connect inorganic with the organic sphere and are therefore affected by multiple environmental and biological drivers, which can respond to climate change. Next to carbon and phosphorus, nitrogen is one of the most important elements in nature, but its transformation processes are more complex compared to the others. It connects a multifaceted organic part with the inorganic sphere. In terrestrial ecosystems soil microbial organisms play a key role in nitrogen cycling as they mediate many of these processes and connect both of these two worlds, the organic and inorganic one. By fixing dinitrogen (N_2) from the atmosphere, the biggest N pool on earth, they produce reactive nitrogen, which can be transferred to plants, where it is incorporated into complex organic nitrogen structures. The decomposition of soil and plant organic material is carried out by soil bacteria and fungi, which first depolymerize high-molecular weight organic nitrogen (HMW-ON) compounds into low-molecular weight organic nitrogen (LMW-ON) compounds such as oligomers and monomers. The latter compounds are taken up by soil microbes to be used as nitrogen and energy (carbon) source. Excess inorganic nitrogen is excreted as ammonium and can be used as energy source by chemolithoautotrophic nitrifiers by oxidation to nitrate, which can serve as terminal electron acceptor by heterotrophic denitrifiers. Ammonium and nitrate can also be used as nitrogen source for plant nitrogen assimilation. With this process the risk for both leaching (nitrifiers) and gaseous nitrogen losses from soils (nitrifiers and denitrifiers) increases.

With progressing climate change global temperatures are expected to rise well above 1.5 °C by 2050 compared to pre-industrial levels, independently from reduction of greenhouse gas emissions or changing socioeconomic pathways (IPCC, 2021). Besides an increase in global greenhouse gases like carbon dioxide (CO_2) which can have important implications for the growth of plants through the CO_2 fertilization effect (Donohue et al., 2013; IPCC, 2001) and also microorganisms (Zak et al., 2000; Drissner et al., 2007), rising temperatures are expected to change the environmental conditions for plants and microbes and to alter biogeochemical cycles. As rising air temperatures trigger increases in soil temperature, soil decomposition processes are expected to be affected. But not only direct, physical consequences are expected as with an increase in soil temperature transformation processes in biogeochemical cycles like in the nitrogen cycle are expected to be altered. These impacts of warming can be manifold: The correlation of elevated temperatures and soil

nitrogen availability (Dawes et al., 2017; Melillo et al., 2011), generally indicates a change in nitrogen input and output rates (Olson, 1958) and lie the foundation for the opening of the nitrogen cycle due to increased nitrogen losses. This change in nitrogen availability can impact the nitrogen use efficiency (NUE) of microbes, altering decomposition (Mooshammer et al., 2014), and the share of organic nitrogen channeled into the inorganic nitrogen cycle. Soil nitrogen transformation processes in the inorganic nitrogen cycle, like nitrification and denitrification, are especially expected to be impacted, either directly through physical warming effects accelerating process rates or indirectly through changing the flux partitioning in the nitrogen cycle (Séneca et al., 2020). While an alteration of extracellular enzyme activity in warmed soils is still a matter of debate, as several studies show positive, neutral or negative responses to warming (Schindlbacher et al., 2015; Bell et al., 2010; Cookson et al., 2007; Feng et al., 2007), net N mineralization and nitrification rates (Melillo et al., 2002; Bai et al., 2013) as well as fine-root biomass (Wang et al., 2021) have been found to increase significantly.

Changes in gross nitrogen fluxes are difficult to measure, based on ^{15}N isotope pool dilution approaches, as they happen on a small scale, are highly heterogeneous dynamic and in constant feedback to other nitrogen processes. This is especially the case when the nitrogen cycle is defined as the successive and partially parallel arrangement of fluxes between soil nitrogen pools. For reasons of simplification we here selected the following key soil nitrogen pools to be of interest: soil organic matter (SOM; here referred to as soil bulk nitrogen), dissolved organic nitrogen (DON), microbial biomass nitrogen (N_{mic}), ammonium (NH_4^+) and nitrate (NO_3^-). These pools are interlinked through the following five transformation processes: HMW-ON depolymerization, immobilization, mineralization, nitrification and denitrification/loss processes, respectively.

Quantifying the natural abundances of the stable nitrogen isotopes, ^{15}N and ^{14}N, holds the potential of detecting small-scale and short-term to intermediate-term alterations in nitrogen fluxes. This derives from the fact that every (microbial) conversion process discriminates more or less strongly against the heavier nitrogen isotope, ^{15}N, leading to isotope fractionation, while physical processes (diffusion in water, leaching, uptake, sorption, desorption) exert a negligible isotope effect (Handley & Raven 1992, Denk et al., 2017).

Many possible factors influence ^{15}N isotope fractionation. Besides the influence of overall soil carbon and nitrogen concentrations in soil, microbial activity (depending on soil pH, labile soil carbon and nitrogen concentrations and microbial biomass), precipitation and soil moisture (Amundson et al., 2003), soil sampling depth (Delwiche & Steyn, 1970) and

soil texture (clay; Craine et al., 2014), ^{15}N isotope fractionation strongly depends on soil temperature (Craine et al., 2014; Högberg, 1997). When microorganisms are subject to increased soil temperature more energy is available which is needed to form and break bonds, a process that is slower if the heavier nitrogen isotope is involved. If climate change brings soil temperature closer to their relative optimum of specific nitrogen cycle processes (Saad & Conrad, 1993) their rate will increase and change the flux partitioning between the multitude of soil nitrogen processes, affecting the natural ^{15}N abundances of the interconnected pools and eventually change the isotope fractionation specific processes. While a single pool is expected to increase/decrease in its ^{15}N abundance due to an altered balance between substrate influx, substrate consumption and product efflux, the whole flux from bulk nitrogen to the inorganic nitrogen forms can behave differently, causing increases in nitrogen losses and an opening of the soil nitrogen cycle. Accelerated decomposition through increased temperature can lead to greater losses of inorganic and gaseous nitrogen forms, which are ^{15}N depleted, as mineralization, nitrification and denitrification increase (Philben et al., 2018). This opening of the nitrogen cycle is reflected in increased soil and ecosystem ^{15}N enrichment in treatments where losses of nitrogen are greater.

In this study we assessed the natural ^{15}N abundances of fine roots, bulk soil nitrogen, dissolved organic nitrogen, microbial biomass, and ammonium and nitrate pools in montane forest soils in the long-term warming experiment in Achenkirch, Tyrol, Austria, across three seasons (spring, summer and autumn). With increasing temperature the nitrogen cycle is expected to accelerate and to become more open, which would be reflected in altered $\delta^{15}N$ values of organic and inorganic soil nitrogen pools as well as in root and microbial biomass in the warming treatment. Moreover, given the different temperature sensitivity of the studied processes we expected to find a change in the nitrogen flux partitioning, causing changes in the relative ^{15}N enrichment or depletion of the different nitrogen pools. For instance, an increasing fraction of ammonium being nitrified would cause increasing ^{15}N enrichment of ammonium, while the product, nitrate, would be ^{15}N depleted, unless a large fraction of nitrate is consumed by denitrifiers. Finally, given increasing nitrate losses, we expected nitrate leaching not to alter the isotopic signature of soil nitrate, while denitrification would cause a ^{15}N enrichment of residual soil nitrate.

3.2. Materials and Methods

Site description

The experimental site is located in Achenkirch, Tyrol, Austria (47°34050´´N; 11°38021´´E), at 910 m a.s.l. in the Northern Alps. Climatic conditions are cool and humid, with mean annual temperature of 6.9 °C and mean annual precipitation of 1506 mm (1992-2012, Zentralanstalt für Meteorologie ZAMG). The site is usually snow covered from November/December until April/May. The forest is approximately 130 years old, situated on dolomite bedrock and is dominated by *Picea abies* with interdispersed *Fagus sylvatica* and *Abies alba*. The forest soil represents chromic cambisols and rendzic leptosols. The plots were established in 2004 and 2008, re-

Fig. 3: Experimental site in Achenkirch.

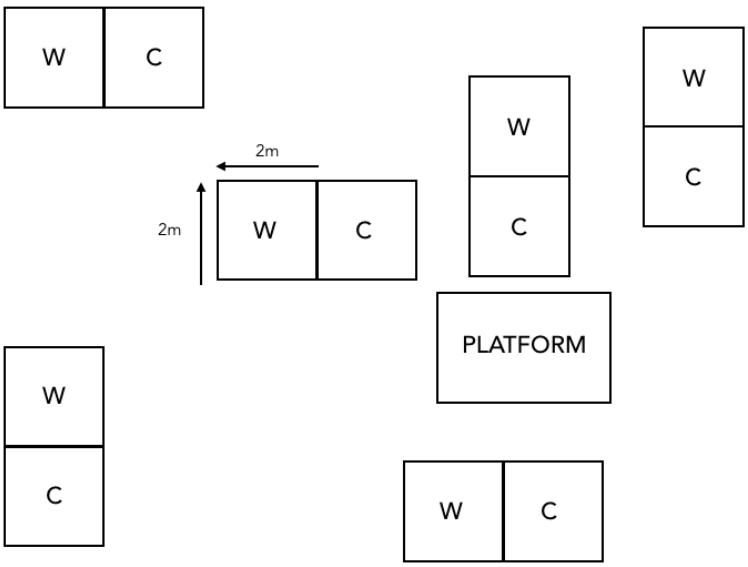


Fig. 4: Organization of experimental site in Achenkirch with warmed (W) and control (C) plots.

spectively, in total consisting of six paired plots, of which six subplots are control plots and six subplots are heated (Fig. 3 and 4). The warmed plots are equipped with resistance heating cables (0.4 cm diameter, TE-CUTE – 0.18 Ohm m^{-1} per UV, Etherma, Austria) at 3 cm soil depth with 7-8 cm spacing between each cable row, warming the soil +4 °C above ambient during the snow-free season (Fig. 5; Schindlbacher et al., 2015). It should be noted, that contrary to similar experiments, in Achenkrich the positive response of soil respiration to warming (~40% increase) was sustained until the time of this study (Schindlbacher & Heinzle, personal communication, 29.11.2021).

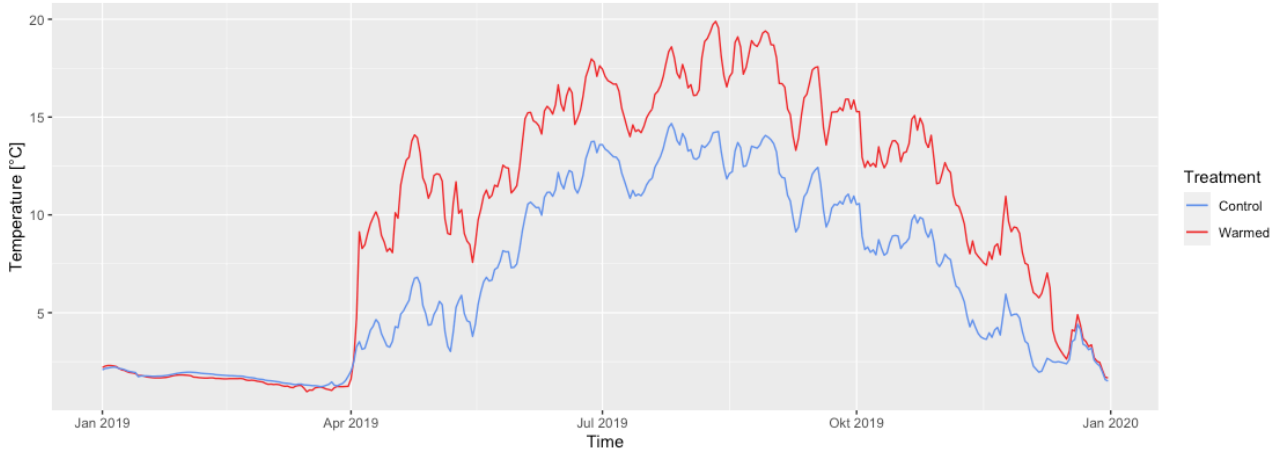


Fig. 5: Temperature Log of warmed and control plots in 2019.

Sampling and measurements

Soils were sampled in May, August and October 2019. In each subplot one composite soil sample was taken with a 2.5 cm diameter corer (5-8 subsamples) from 0-10 cm and 10-20 cm soil depth, leading to 72 soil samples in total. After sieving through 2 mm mesh, fine roots were washed, dried at 60 °C and weighed. Around 4 g of fresh soil aliquots were weighed in for (i) determination of soil water content, (ii) chloroform fumigation extraction (CFE) for microbial biomass nitrogen determination, and (iii) direct extraction. For determination of soilwater content, soil samples were dried in a drying oven (85 °C) and dry mass reweighed. To determine microbial biomass nitrogen soils were fumigated with chloroform in a desiccator for 48 hours. Fumigated and untreated soil samples were then extracted with 30 mL 0.5M K_2SO_4, filtered through ash-free cellulose filters and the extracts stored at -20 °C. In order to measure the natural ^{15}N abundances ($\delta^{15}N$ values) and concentrations of the different labile nitrogen pools, the frozen K_2SO_4 samples were thawed and separated for each measurement procedure to avoid repeated thawing and freezing.

The nitrogen isotopic composition of fine roots and of bulk soil nitrogen were measured by elemental analyzer-isotope ratio mass spectrometry (EA-IRMS), consisting of an EA-Isolink coupled via a ConFlo IV interface to a Delta V Advantage IRMS (Thermo Scientific, Bremen, Germany). For this the dried samples were finely ground in a ball mill (Retsch MM200, Hainau, Germany) and weighed into tin capsules before loading into the autosampler of the EA. The nitrogen isotopic composition of labile soil nitrogen pools (total dissolved nitrogen, TDN, for DON, N_{mic}, NH_4^+ and NO_3^-) was measured using purge-and-trap IRMS (PT-IRMS) after converting into N_2O. The PT- IRMS instrument consisted of a Gasbench II headspace gas analyzer with an integrated cryotrap for N_2O pre-concentration, which was coupled to a Delta V Advantage IRMS (Thermo Scientific, Bremen, Germany).

The isotopic composition of NH_4^+ was determined through microdiffusion followed by the BrO^--azide method. The isotopic composition of NO_3^-, TDN/DON and CFE/N_{mic} followed the VCl_3-azide method, which converts NO_3^- to N_2O. However, for TDN/DON and CFE/N_{mic} preparatory steps were required before the VCl_3-azide method was applicable, involving the alkaline persulfate digestion of organic nitrogen forms and of NH_4^+ to NO_3^-.
To determine DON, TDN was measured and later corrected for the contributions of inorganic nitrogen forms (NH_4^+ and NO_3^-). Here, the untreated K_2SO_4 extracts underwent an alkaline persulfate digestion to transform all nitrogen forms to NO_3^-. For this, 44.04 g $Na_2S_2O_8$, 16.8 g NaOH and 30 g H_3BO_3 were dissolved in 1 L deionized water. Samples and persulfate reagent were mixed in a 1:1 ratio in 1.8 mL HPLC glass vials. In addition, an amino acid dilution series (1 mM N L^{-1}, 1:2 dilution - 500 µM - 3.9 µM - 0 µM) was prepared in 0.5 M K_2SO_4. Samples and amino acid standards were autoclaved for 60 min at 120 °C. An additional external nitrate standard series (KNO_3, 500 µM - 3.9 µM) was prepared in 0.5 M K_2SO_4, persulfate digested and measured to determine the digestion efficiency by comparing it to the amino acid dilution series. Isotopic calibration of this method was performed using the standards presented in Table 2.

Table 2: Isotope standards (15 μM) for $\delta^{15}N$-determination of NO_3^- and NH_4^+-samples.

TDN & CFE (NO_3^- converted)	USGS 40 $\delta^{15}N$ = -4.52	USGS 41 $\delta^{15}N$ = +47.57	USGS 73 $\delta^{15}N$ = -5.21	USGS 74 $\delta^{15}N$ = 30.19	USGS 75 $\delta^{15}N$ = 61.53
NO_3^-	Std 1 $\delta^{15}N$ = -1.8	Std 2 $\delta^{15}N$ = -2.8	Std 3 $\delta^{15}N$ = +4.7	Std 4 $\delta^{15}N$ = -35.2	-
NH_4^+	IAEA 1 $\delta^{15}N$ = +0.4	IAEA 2 $\delta^{15}N$ = +20.3	USGS 25 $\delta^{15}N$ = -30.4	-	-

For N_{mic} determination the CFE-K_2SO_4-extracts were also digested in the autoclave with persulfate reagent as depicted above and had amino acid concentration and isotope standards as well as external KNO_3^- standards added. The resulting NO_3^- from persulfate digestions as well as NO_3^- from the undigested K_2SO_4-extracts were converted to N_2O by using the VCl_3-azide method. For this, the NO_3^- of the respective samples was converted to N_2O in two steps: Vanadium (III) chloride (VCl_3) reduced the NO_3^- to NO_2^-, which was then further reduced to N_2O by acidic sodium azide (Lachouani et al., 2010). Related isotope calibration standards are presented in Table 2. Aliquots (500 µL) of the respective samples (TDN, CFE or undigested K_2SO_4-extract for NO_3^-) were pipetted into 12 mL exetainers, closed and purged with helium for 10 minutes. After this, 100 µL of sodium azide buffer (1.3 g NaN_3 in 10 mL Milli-Q, mixed 1:1 with 10 % acetic acid, flushed with helium for one hour) was injected into the exetainers with a gas-tight syringe to avoid air bubbles. Subsequently, 500 µL of VCl_3-solution (0.79 g VCl_3 in 100 mL 3.2 % HCl) was added to the sample, mixed on a vortex and incubated at 37 °C for 24 hours. To stop the reaction, 150 µL of 6 M NaOH were injected to neutralize the NaN_3. The produced N_2O was transferred to empty, vacuumed exetainers with helium to avoid clogging of the PT-IRMS needle.

$\delta^{15}N$ signatures of NH_4^+ were determined through microdiffusion with acid traps at alkaline solution pH followed by the BrO^--azide method (Zhang et al., 2015). For microdiffusion, acid traps were prepared made of Teflon tape enclosing a cellulose disc, on which 4 µL 2.5 M $KHSO_4$ was pipetted, before sealing them. Then, 0.1 g MgO was weighed in 20 mL scintillation vials, before adding 10 mL of the K_2SO_4 extracts and one acid trap each. The vials were placed on a shaker at 37 °C for a three days. After the incubation, the scintillation vials were opened and acid traps removed and transferred into 1.5 mL reaction tubes for drying in a desiccator with concentrated sulfuric acid for three days. The dry discs were then removed from the Teflon cover, NH_4^+ dissolved in 1 mL Milli-Q and placed on a shaker for 30 min. In the meantime, 1 mL K_2SO_4 blanks (0.5 M), Milli-Q blanks, NH_4^+ concentration standards (50 - 1.56 µM NH_4Cl), and NH_4^+ isotope standards (15 µM, see Table 2) were prepared and transferred to 12 mL exetainers. The sample extracts were diluted according to previously measured photometric concentration data in order to reach a final NH_4^+ concentration of 20 µM and also pipetted into exetainers (1 mL).

To oxidize NH_4^+ to NO_2^-, a hypobromite solution (BrO-) was made. For this, 0.2 mL stock solution (0.12 g sodium bromate and 1 g sodium bromide in 50 mL MQ) was filled up to 10 mL with MQ. Adding 0.6 mL 6 M HCl started the reaction. After 5 minutes, 10 mL of 10 M NaOH was added to stop the reaction. Then, 0.1 mL of this hypobromite working solution

was added to all exetainers as an oxidation catalyst. After 30 min 33 µl sodium arsenite solution (0.51 g $NaAsO_2$ in 10 mL Milli-Q) was added to quench the reaction. To further convert NO_2^- to N_2O, 0.15 mL NaN_3 (1.3 g NaN_3 in 10 mL Milli-Q, mixed 1:1 with 10 % acetic acid, flushed with helium for one hour) was injected into the closed exetainers with a gas-tight syringe. After 30 min, the reaction was stopped by addition of 0.15 mL 10 M NaOH. Again, the N_2O produced was transferred to a new, flushed and evacuated set of exetainers to determine the isotopic ratios by PT-IRMS.

Isotope notation and isotope modeling

Natural ^{15}N abundances are presented in the delta notation as follows:

$$\delta^{15}N\ (‰) = [(^{15}N_{sample}/^{14}N_{sample})/(^{15}N_{standard}/^{14}N_{standard}) - 1] \times 1000$$

Isotope ratios are reported relative to air (atmospheric dinitrogen, N_2) as the international accepted isotope standard.

In (semi-)closed systems one can calculate the isotopic compositions of residual substrate (RS) and cumulative product (CP), depending on the fraction f of substrate converted to product, the isotope fractionation of the process (Δ) and the isotopic composition of initial substrates (IS, δ_{input}) using the following Rayleigh isotope fractionation equations:

$$\delta_{RS} = \delta_{input} - \Delta * ln(1 - f)$$

$$\delta_{CP} = \delta_{input} + \Delta * \frac{(1 - f)}{f} * ln(1 -)$$

This was reiterated for each successive process in the connected model of the soil nitrogen cycle, where CP of a previous process becomes the IS of the consecutive process. For more details please refer to Xu et al. (2021). Finally, microbial nitrogen use efficiency (NUE) was calculated based on bulk soil nitrogen concentrations (N_{bulk}) and the estimated fractions f of substrate converted by the specific processes i.e. depolymerization, microbial uptake and mineralization according to the following equation:

$$NUE = \frac{N_{bulk} * f_{depoly} * f_{uptake} - N_{bulk} * f_{depoly} * f_{uptake} * f_{min}}{N_{bulk} * f_{depoly} * f_{uptake}}$$

Data evaluation and statistics

The respective PT-IRMS $\delta^{15}N$-output of TDN, CFE, NH_4^+ and NO_3^- samples was blank- and isotope-corrected. $\delta^{15}N$ values of DON, N_{mic}, NH_4^+ and NO_3^- were calculated based on concentration weighted calculations. $\delta^{15}N$ values of fine roots and bulk soil nitrogen were added to the combined data set. After outlier corrections, testing for normality and data transformation of $\delta^{15}N$ data, Linear Mixed Effects Regression (*lmer*) models of each pool were calculated in R to check statistical significance and the effect of treatment and season and their interaction (Pinheiro et al., 2019; Kuznetsova et al., 2017; Bates et al., 2015; Barton, 2020; Hyndman et al., 2020). Values were considered significant at $p < 0.05$. Results were visualized with *ggplot* (Wickham, 2016).

In addition, a Principal Component Analysis (PCA) was conducted to get a more general picture of the correlations in the respective soil nitrogen pools (Kassambara & Mundt, 2020).

3.3. Results

The concentrations of bulk soil nitrogen, fine roots, N_{mic}, NH_4^+ and NO_3^- were not significantly different when tested for the contrast of warming and control treatments except for DON, which decreased in warmed soils (Fig. 1). Seasonality had a significant effect on the nitrogen concentrations of fine roots ($p < 0.001$), DON ($p < 0.001$), N_{mic} ($p < 0.001$) and NO_3^- ($p < 0.001$, Table S1). In the case of DON, however, the interaction term of season and treatment was also significant, indicating that warming had a stronger negative effect on DON in May than in October 2019.

According to the *lmer*-models the $\delta^{15}N$ values of all measured pools, bulk soil nitrogen ($p = 0.0015$), roots ($p < 0.001$), DON ($p < 0.001$), N_{mic} ($p < 0.001$), NH_4^+ ($p < 0.001$) and NO_3^- ($p < 0.001$), were statistically significantly affected by season (Fig. 6, Table 3). Warming only significantly affected the $\delta^{15}N$ values of fine roots ($p < 0.001$) and NH_4^+ ($p = 0.0022$), with ^{15}N enrichment in warmed soils (see Fig. S4 for combined $\delta^{15}N$ data).

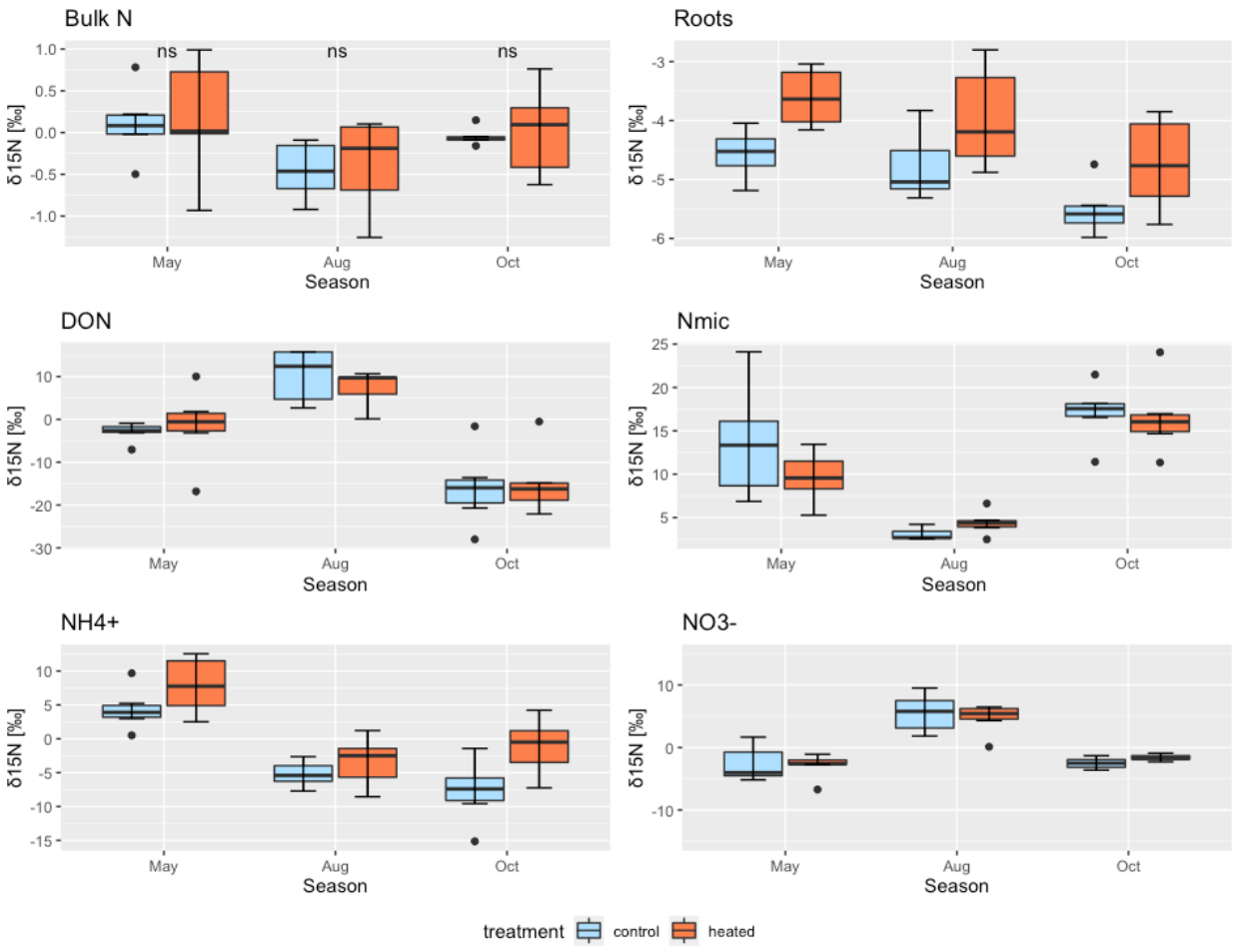


Fig. 6: $\delta^{15}N$ values of heated and control plots for different nitrogen pools in the soil over three seasons of sampling.

Table 3: Summary of statistical significance of lmer models for $\delta^{15}N$ and lmer models/Kruskal-Wallis for concentration values in different pools of soil nitrogen and fine roots. If pool data were transformed, transformation type is indicated.

	Factor	Bulk N	Roots	DON	N_{mic}	NH_4^+	NO_3^-
Transformation		Squareroot	BoxCox	-	BoxCox	-	Log
$\delta^{15}N$ values	Treatment	n.s.	***	n.s.	n.s.	**	n.s.
	Season	**	***	***	***	***	***
	Interaction	n.s.	n.s.	n.s.	*	n.s.	n.s.
Transformation		-	BoxCox	BoxCox	BoxCox	-	-
Concen-tration	Treatment	n.s.	n.s.	**	n.s.	n.s.	n.s.
	Season	n.s.	***	***	***	n.s.	*
	Interaction	-	n.s.	**	n.s.	-	-

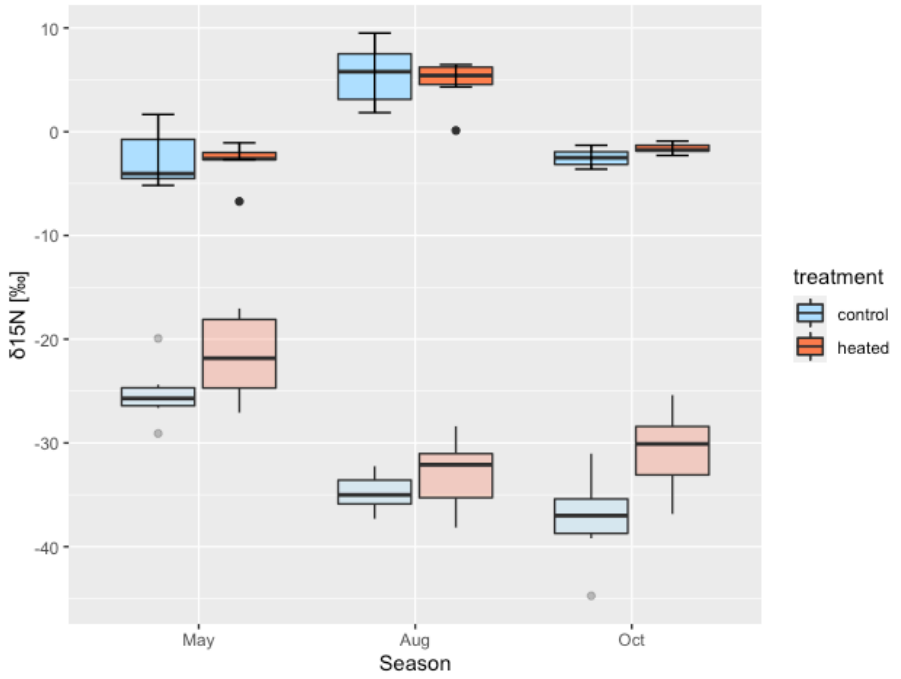


Fig. 7: Calculated (transparent, lower boxplots) and measured (opaque, upper boxplots) $\delta^{15}N$ values of the NO_3^- pool. Measured nitrate data is much more ^{15}N enriched than expected according to calculations.

The ^{15}N enrichment of soil NH_4^+ pool has important implications for the interpretation of nitrification as NO_3^- was not affected by soil warming. Quantifying and modeling the isotope fractionation (^{15}N enrichment or depletion) from ammonium to nitrate is therefore of importance to identify the pathways of soil nitrogen losses. Fig. 7 shows calculated $\delta^{15}N$ values according to isotope fractionation and measured $\delta^{15}N$ values of the nitrate pool. Calculated nitrate values were based on the subtraction of the average isotope fractionation factor of 29.6 ‰ for nitrification according to Denk et al. (2017). Measured $\delta^{15}NO_3^-$ values were much higher than expected according to nitrification.

Table 4: Calculated apparent isotope fractionation values ($\delta^{15}NO_3^-$ - δ^{15} NH_4^+) of the nitrification process. The values strongly deviate from the expected isotope fractionation factors.

∆ nitrification	Heated	Control
May	-10.8	-7.0
August	8.1	10.7
October	2.8	10.2

Table 4 shows the mean apparent isotope fractionation factors of nitrification as the difference between measured $\delta^{15}N$ signatures of NH_4^+ and NO_3^- for warmed and control soils for the three sampling dates May, August and October 2019. No one of the values reached the expected isotope fractionation factor of -29.6 ‰ of nitrifiers but rather show a less strong depletion than anticipated, due to the strong impact of denitrifiers ^{15}N enriching the soil NO_3^- pool.

A Principal Components Analysis (PCA) also highlighted less warming treatment effects than seasonal effects. Both axes of the PCA explained 72.8% of the total variance for treatment and for seasonality (Fig. 8). In addition, microbial NUE was not significantly affected by long-time soil warming. Rather a seasonal change in the warming effect was visible, however this was not significant (Fig. S2).

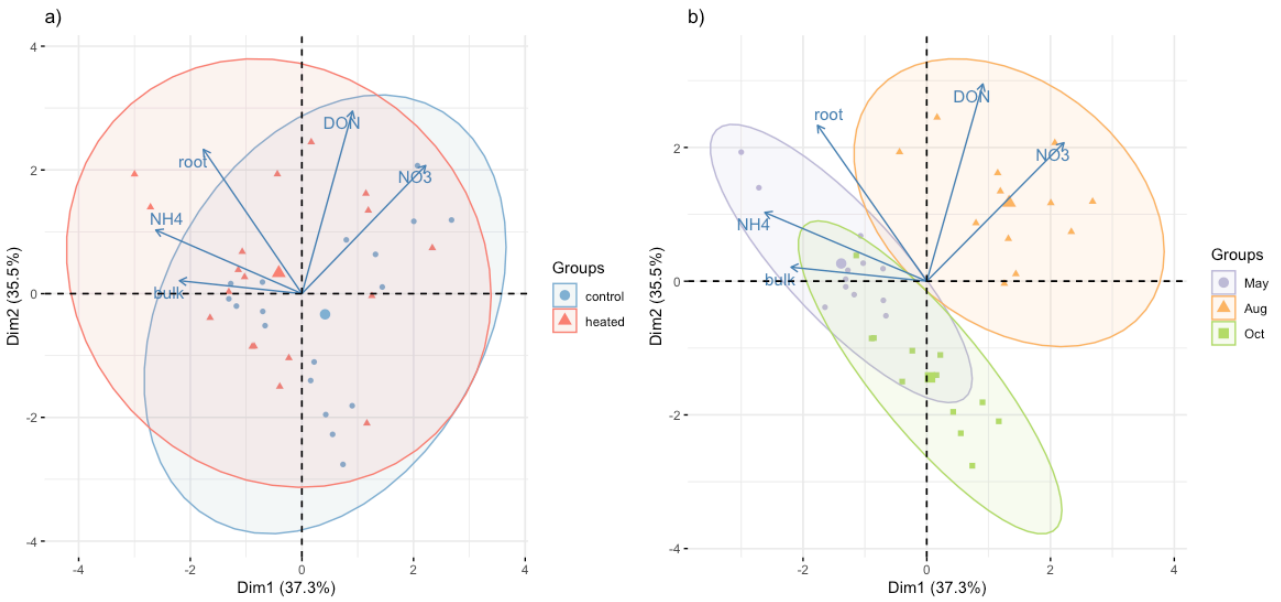


Fig. 8: Principal components analysis of $\delta^{15}N$ values of soil nitrogen pools highlighted for a) treatment and b) seasonal data.

3.4.Discussion

In the Achenkirch warming experiment we expected to find changes in $\delta^{15}N$ values of various soil nitrogen pools, especially in the inorganic nitrogen pools, caused by long-term soil warming. Isotopic alterations due to the warming treatment would signify a change in the nitrogen flux partitioning and indicate an opening of the soil nitrogen cycle. The results generally showed (i) a strong seasonal effect on both, $\delta^{15}N$ signatures and concentrations, but less of a treatment effect on both across all measured nitrogen pools. While, in this case, evaluating concentration values does allow to detect or quantify nitrogen losses via isotope fractionating pathways (denitrification) or nitrification followed by leaching (NO_3^- leaching), the isotopic composition of the pools allows to gain deeper insights into warming effects on soil nitrogen cycling and nitrogen losses. In line with this, (ii) the soil ammonium pool and fine roots showed a significant and seasonally consistent increase in their $\delta^{15}N$ values in warmed plots, suggesting an opening of the nitrogen cycle. Connecting these two findings with existing data of nitrogen losses allowed us to (iii) put these findings in a global climate change context.

Warming effects on soil mineralization and nitrification

Among the studied soil nitrogen pools, fine roots and soil NH_4^+ were significantly ^{15}N enriched in the warming treatment. These two pools are inherently connected as plants and their roots take up inorganic nitrogen and therefore isotopically mirror underlying soil inorganic nitrogen transformation processes on an intermediate temporal scale. This refers to plants predominantly meeting their nitrogen demand via uptake of available inorganic soil nitrogen sources, with little contributions from labile organic nitrogen pools. The increase in fine root $\delta^{15}N$ therefore clearly indicates an increased fraction of ammonium being nitrified, as well as an increased fraction of nitrate being denitrified, overall causing a ^{15}N enrichment of the inorganic soil nitrogen pool in warmed soils.

Considering the soil ammonium pool, according to the Rayleigh isotope fractionation theory, NH_4^+ not only acts as the cumulative product of mineralization of organic nitrogen, but also as initial substrate for nitrification. Hence, the ^{15}N abundance of the ammonium pool is determined by the two processes of mineralization and nitrification, with NH_4^+ as cumulative product, and initial substrate, respectively. However, since the preceding soil nitrogen pools, especially the N_{mic} pool, did not show any treatment effect it is more likely that ins-

tead of a control through the process of mineralization, the ammonium pool was isotope fractionated through nitrification. Increased $\delta^{15}N$ values in the ammonium pool therefore suggest an increased fraction of ammonium consumed by nitrifiers in warmed soils.

Besides temperature, precipitation (soil water availability) is an important factor controlling ecosystem and soil nitrogen cycling. In ecosystems these two factors, temperature and moisture, usually strongly influence each other. With continuous soil warming during the vegetation season, soil moisture is reduced (Xu et al., 2013), which was also found in Achenkirch, though high MAP and frequent precipitation events reset these differences regularly. Several studies have shown that drier ecosystems or drier soil conditions cause a ^{15}N enrichment of plants and soils, indicating an opening of the nitrogen cycle (Austin & Vitousek, 1998; Amundson et al., 2003; Craine et al., 2014).

However, it needs to be considered that temperature and water availability are not the only factors influencing natural ^{15}N abundances of nitrogen pools in terrestrial ecosystems, but that other ecosystem properties, like availability of labile soil carbon, nitrogen, and phosphorus, their stoichiometric ratio, microbial community composition and activity, and soil texture, can also impact nitrogen isotope fractionation. In the context of studies at the same Achenkirch experiment several studies and data sets allow to tackle this issue. Concerning nitrogen availability for example, Shi (2019) found increased SOM decomposition in warmed soils, which contributed to increased nitrogen availability while leading to an increase in phosphorus deficiency and thereby increased microbial carbon-phosphorus co-limitation. Control soils were mainly characterized by microbial carbon limitation. The increase in carbon-phosphorus co-limitation in warmed soils would explain that nitrogen was not limiting to the soil microbial communities and that this excess of nitrogen was amenable to losses from soils. In a global meta-analysis of gross nitrification rates in soil Elrys et al. (2021) synthesized that gross nitrification rates increase with bulk soil nitrogen and decrease with soil C:N ratios, and that precipitation and temperature affect gross nitrification rates by altering soil C:N and bulk soil nitrogen. Bulk soil nitrogen and temperature increased heterotrophic nitrification rates, while soil C:N and soil pH controlled autotrophic nitrification rates. This stands in sharp contrast to Craine et al., (2014), who found in a global meta-analysis that soil $\delta^{15}N$ decreases with increasing soil nitrogen content, indicating less nitrogen losses, which are largely controlled by nitrification promoting leaching or denitrification losses at higher soil moisture. However, the same study found no direct relationship between climate and soil $\delta^{15}N$ based on structural equation models. Another factor of interest regards microbial community composition, as microbes are the key mediators of the inorganic nitrogen cycle and therefore control ammonification, nitrification and denitrifi-

cation processes. In the case of the Achenkirch warming experiment, however, medium-term and long-term warming caused no significant shift in microbial composition based on phospholipid fatty analysis (Schindlbacher et al., 2011), amplicon sequencing (Kuffner et al., 2012) and metaproteomics (Liu et al., 2017) and no thermal adaptation, most likely because of a strong microbial heterogeneity (Schindlbacher et al., 2015). However, a slight change in microbial functionality at the same experimental site was detected and linked to elevated CO_2 efflux based on the meta-proteomics approach (Liu et al., 2017). Adding to this, a global meta-analysis conducted by Bai et al. (2013) found evidence for significant increases in net mineralization and net nitrification under experimental warming, indicating a higher potential for nitrogen losses from warmed soils. Except for organic nitrogen mineralization, the results align with our finding. This adds substantially more weight to warming causing crucial changes in the soil nitrogen cycle and promoting nitrogen losses from soils.

Warming effects on nitrate losses

In principle nitrogen losses in pristine forests can come from hydrological losses of DON and nitrate, or from denitrification of nitrate via gaseous losses (Davis, 2014; Hedin et al., 1995; Gundersen et al., 2006). The isotopic alterations in the soil NH_4^+ and NO_3^- pools strongly indicate elevated nitrification and increased nitrate losses through denitrification in warmed soils, while leaching of DON seems to play a minor role, with average concentrations across all seasons of extractable DON, ammonium and nitrate of 10.29, 3.0 and 22.66 µg N g^{-1} soil DW. According to Rayleigh isotope fractionation, and as indicated by Denk et al. (2017), nitrification leads to a ^{15}N enrichment of the soil ammonium pool, while soil nitrate is ^{15}N depleted, on average by 29.6‰, given that nitrate is not consumed by consecutive isotope fractionating processes. Our results clearly showed a ^{15}N enrichment of the ammonium pool caused by a greater fraction of ammonium being nitrified in warmed soils, but also a strong ^{15}N enrichment of the soil nitrate pool, in August and October 2019 being even higher in $\delta^{15}N$ as in ammonium (Fig. 6 and 7), which was not significantly affected by soil warming. In May soil was ^{15}N depleted relative to ammonium by 7 to 11‰, which is expected due to isotope fractionation by nitrification, though much less than expected by average isotope fractionation by archaeal and bacterial ammonia oxidizers of ~ 30‰ (Denk et al., 2017; Mooshammer et al., 2020; Liu et al., 2021). In August and October the $\delta^{15}N$ values of nitrate were even ^{15}N enriched relative to soil ammonium by 3 to 11 ‰ (Table 4). Without further nitrate consumption by denitrifiers or dissimilatory nitrate redu-

cers to ammonium, nitrate would be lost by hydrological pathways, where leaching as a physical process would not fractionate against ^{15}N, i.e. nitrate would be exported as produced as produced isotopically by nitrifiers. Though, if nitrate leaching would be quantitatively important, this would cause losses of ^{15}N depleted nitrate and ^{15}N enrichment of nitrogen residing in the system as soil organic matter but no ^{15}N enrichment of nitrate relative to ammonium. This indicates a strong opening of the nitrogen cycle but different mechanisms dominating across seasons, with predominant denitrification losses, which increased from spring to summer and autumn in this study.

This follows what Koba et al. (2012) postulated, namely that „the balance between gaseous N loss with strong ^{15}N discrimination and discharged N loss with slight or null discrimination determines the net effect of ^{15}N discrimination“ on soil nitrate and on the ecosystem level. Overall, nitrogen is preferentially lost as ^{14}N from the ecosystem, either through nitrate leaching or denitrification, enriching the corresponding soil nitrogen pool with ^{15}N (Garten et al., 2007; Craine et al., 2014). In the Achenkirch warming experiment nitrogen losses were quantified as soil water NO_3^- and soil N_2O emissions. DNRA was not quantified yet, but likely contributes little to nitrate dissimilation. If the DNRA process increased in warmed soils this would be reflected in elevated ammonium concentrations and decreased $\delta^{15}N$ values as DNRA bacteria strongly discriminate against ^{15}N (Asamoto et al., 2021).

According to Borken (personal communication, 25.11.2021) NH_4^+ could not be identified in soil water samples collected by suction cups, while significant concentrations of NO_3^- were detected, though not differing between control and warmed soils. Annual leaching losses of 300-500 g NO_3^- m^{-2} yr^{-1} were determined in Achenkirch, though the soil hydrological model needs to be improved. Gaseous N_2O losses were quantified by Schindlbacher and Heinzle (personal communication, 29.11.2021). While season exerted a significant effect on soil N_2O emissions ($p = 0.0312$), no warming treatment effect was detected in the long-term, but these fluxes are spatio-temporally highly variable obviating treatment effects. Also, N_2O measurement is highly susceptible to losses and therefore difficult to be carried out *in-situ* which might contribute to non significant findings.

However, elevated $\delta^{15}N$ values of the nitrate pool strongly suggest significant nitrogen losses through denitrification, not by leaching of nitrate. The finding of dominant denitrification losses of nitrate from warmed soils is in accordance with Elrys et al. (2021), who found that gross nitrification rates were positively related to soil nitrous oxide emissions, given that soil nitrifiers emit NO and N_2O and that they provide the substrate to denitrifiers. However, as previously mentioned, nitrate $\delta^{15}N$ values were not significantly different bet-

ween control and warmed soils, though those of ammonium were. Overall, this highlights that soil nitrogen (nitrate) losses were dominated by denitrifiers in both conditions, but that the increase in $\delta^{15}N$ values of fine roots and ammonium indicates that in warmed soils greater overall nitrogen losses are caused by increased nitrate leaching. Booth et al. (2005) showed that gross nitrification strongly depends on gross nitrogen mineralization, though this was not evident in this study in warmed soils. On the other hand we did not find a consistent decrease in microbial nitrogen use efficiency in warmed soils, which would indicate that less organic nitrogen, which is taken up by soil microbes will be invested in microbial growth and more of this being mineralized. Microbial nitrogen use efficiency decreased in spring and increased in autumn in warmed soils, though non-significantly (Table S2, Fig. S2). Microbial NUE is considered a valve that directs the amount of organic nitrogen flowing into the inorganic nitrogen cycle through ammonification and controls the nitrogen conservation in soils (Mooshammer et al., 2014).

Seasonal effects

The strong seasonal effect on **concentrations** of soil and fine root nitrogen pools is related to seasonal changes in plant phenology and activity, microbial activity and soil microclimate (temperature and soil moisture). Such a short-term variation in soil biogeochemistry across the vegetation season has been discussed in previous literature (e.g. Arheimer et al., 1996). We found significant seasonal changes in the concentrations of all studied nitrogen pools except for bulk soil nitrogen and NH_4^+ (Fig. S1). While fine root nitrogen and microbial biomass nitrogen peaked in summer, DON decreased with season, ammonium remained relatively constant and nitrate increased. In contrast, Heinzle et al. (2021), using a microdialysis approach to estimate diffusive fluxes of the same nitrogen forms, found a decrease in diffusive nitrate fluxes, an increase in diffusive ammonium fluxes and free amino acid fluxes peaking in August 2019. This clearly shows that different ways to approach soil nitrogen availability, here diffusive fluxes versus extractable pools, can produce very dissimilar proxies of nitrogen availability. However, in accordance with Heinzle et al. (2021), the concentrations of the respective inorganic nitrogen pools were not affected by soil warming. Only DON exhibited a significant interaction of season and treatment, indicating that warming reduced DON in spring but did not affect DON in autumn. This followed increased mineralization of DON in spring, as reflected by increased soil ammonium values in spring but not in autumn.

Considering the **$\delta^{15}N$ values** of the respective nitrogen pools provides further insights into the underlying soil nitrogen processes. All nitrogen pools showed highly significant seasonal changes, but not the same ones in terms of seasonality. Fine roots showed a decline in $\delta^{15}N$ with season, implying increased ammonium uptake towards later seasons and a decrease in root nitrate uptake. DON peaked during summer, likely caused by drought-induced promotion of microbial turnover, as soil microbial nitrogen was ^{15}N enriched throughout all seasons. In contrast, microbial biomass nitrogen showed the lowest ^{15}N enrichment in summer, indicating high assimilations of organic nitrogen (a non-fractionating process) and low dissimilatory processes, which would cause high ^{15}N fractionation (ammonification causing ammonium to be ^{15}N depleted and microbial biomass to become ^{15}N enriched) (Collins et al., 2009). Spring and autumn microbial biomass nitrogen was highly ^{15}N enriched, caused by high dissimilatory processes and less assimilatory processes. Overall, the natural ^{15}N abundance of ammonium decreased with season, highlighting a decreased fraction of ammonium being nitrified, while the natural ^{15}N abundance of soil nitrate peaked in summer, showing the maximal fraction of nitrate being denitrified during summer, even though the soils were relatively dry. However, anoxic or suboxic microsites can still occur in microaggregates, allowing denitrifiers to thrive even under relatively dry soil conditions. Seasonal changes in and warming effects on plant $\delta^{15}N$ values caused by isotopic alterations in soil available nitrogen have also been studied at the alpine treeline (Dawes et al., 2017). The latter authors showed higher foliar $\delta^{15}N$ values and a transient increase in soil inorganic N being indicative of a persistent increase in plant-available N and greater soil nitrogen losses in warmer soils.

3.5. Conclusion

An altered carbon cycle has far reaching consequences for the global climate. As global biogeochemical cycles are inherently connected, an acceleration of one cycle almost always leads to the alteration of others. Faster carbon cycling, increasing atmospheric CO_2 and global warming can therefore influence the nitrogen cycle. The balance of nitrogen cycling on terrestrial ecosystems is of utter importance as it guarantees ecosystem stability, a resilient biodiversity and ecosystem services.
In this study we found three major points which are all related to seasonal changes and warming effects on nitrogen flux partitioning, thus affecting the isotope fingerprints of the different nitrogen pools:

(i) Increased ^{15}N enrichment of soil ammonium shows that nitrification was accelerated in warmed soils causing a larger fraction of ammonium being oxidized by nitrifiers. An increase in nitrification is clearly and directly linked to an increased risk of nitrogen losses through nitrate leaching or denitrification.
(ii) Denitrification rates were generally large as in summer and autumn $\delta^{15}N$ signatures of nitrate were higher than those of soil ammonium, showing that a large fraction of nitrate was consumed by denitrifiers, followed by gaseous nitrogen losses as NO, N_2O and N_2.
(iii) Roots integrate the nitrogen isotopic composition of soil inorganic nitrogen (and a bit of organic bio-available nitrogen) over a longer time period (months) compared to inorganic nitrogen itself (days), and therefore provide a valid proxy of the medium-term changes in the isotopic composition of inorganic nitrogen. Roots clearly indicated a ^{15}N enrichment of soil inorganic nitrogen, caused by increased nitrogen losses and due to a more open nitrogen cycle in warmed soils.

This study highlights the potential for unprecedented insights into environmental change effects, like soil warming, on the terrestrial nitrogen cycle based on a natural ^{15}N abundance approach. The depth of insight could hardly be reached with conventional techniques of ^{15}N isotope pool dilution, soil gas efflux measurements (almost impossible e.g. for N_2 from denitrification) and leachate studies. Using ^{15}N isotope pool dilution also invokes sample processing (sieving, ^{15}N substrate addition) while this natural ^{15}N abundance approach is non-invasive as it characterizes the *status quo* of soil nitrogen cycling at the time of sampling. This work and future ^{15}N-isotope studies do not only show a

great possibility to further consolidate existing knowledge about fluxes and processes in the nitrogen cycle but also hold the potential to generate universal information about effects of global warming. Especially global meta-analyses allow conclusions on the development of nitrogen cycles in different ecosystem types during climate change through isotopic pattern analysis, as high mountain forests are rare and specific examples for global ecosystems. Being aware of transformations in biogeochemical cycles and recognizing possible tipping points of ecosystems are important tools to estimate the impact of climate change and act upon the gained knowledge to tackle potentially devastating consequences.

3.6. Supplementary Material

Table S1:

pool	$\delta^{15}N$ values					concentration values [µg/g DM]				
	mean	std deviation	lmer			mean	std deviation	lmer/Kruskal Wallis		
			treatment	season	interaction			treatment	season	interaction
Coarse litter	-6.9603	0.8163	0.6366	0.8848	0.6350	7776.66	2596.96	0.3267	0.2535	-
Fine litter	-5.3751	0.4831	0.8351	0.0920	0.8257	10863.9	2605.8	0.5064	<0.0001	-
Bulk N	-0.0962	0.5084	0.926	0.0015	0.9978	7852.2	1794.9	0.1131	0.6505	-
Roots	-4.5292	0.8443	<0.0001	<0.0001	0.6517	9594.64	1714.5	0.8786	<0.0001	0.5594
DON	-2.9201	11.897	0.9465	<0.0001	0.6257	12.468	8.0550	0.0057	<0.0001	0.0057
Nmic	10.728	6.5411	0.9733	<0.0001	0.0258	51.734	48.886	0.4127	<0.0001	0.3412
NH4+	-0.8623	6.4592	0.0022	<0.0001	0.2622	2.9530	2.6199	0.7517	0.111	-
NO3-	0.0899	4.1514	0.965	<0.0001	0.574	22.663	11.144	0.6578	0.02589	-

Table S2:

	Season	Treatment	Interaction
NUE lmer	0.1238	0.9802	0.1679

Table S3:

$\delta^{15}N$ values		Beech			Spruce		
	Season	Leaf	Bark	Trunk	Leaf	Bark	Trunk
Mean	**May**	-5.346	-5.355	-3.186	-7.452	-6.849	-7.549
	Aug	-5.515	-5.489	-2.310	-7.962	-7.535	-4.224
	Oct	-5.159	-4.949	-3.486	-7.549	-7.330	-5.327
	May-Oct	-5.340	-5.264	-2.994	-7.654	-7.238	-5.700
Standard deviation	**May**	0.680	0.747	0.992	0.986	2.639	2.949
	Aug	0.425	0.731	2.051	0.658	0.550	2.831
	Oct	0.808	0.965	0.810	0.623	0.630	1.420
	May-Oct	0.635	0.807	1.409	0.761	1.531	2.745

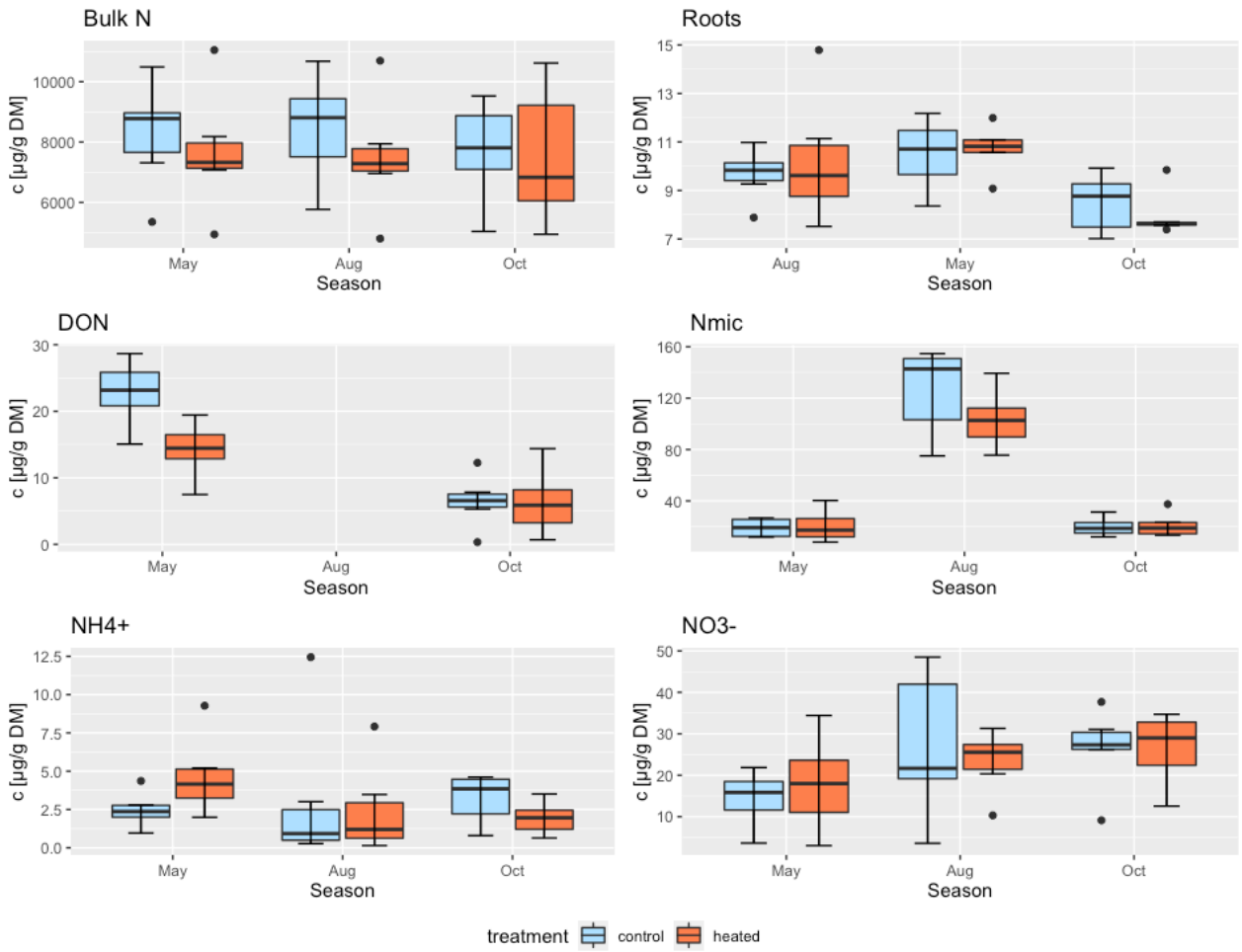


Fig. S1: Concentration pool plots for heated and control treatment over three seasons.

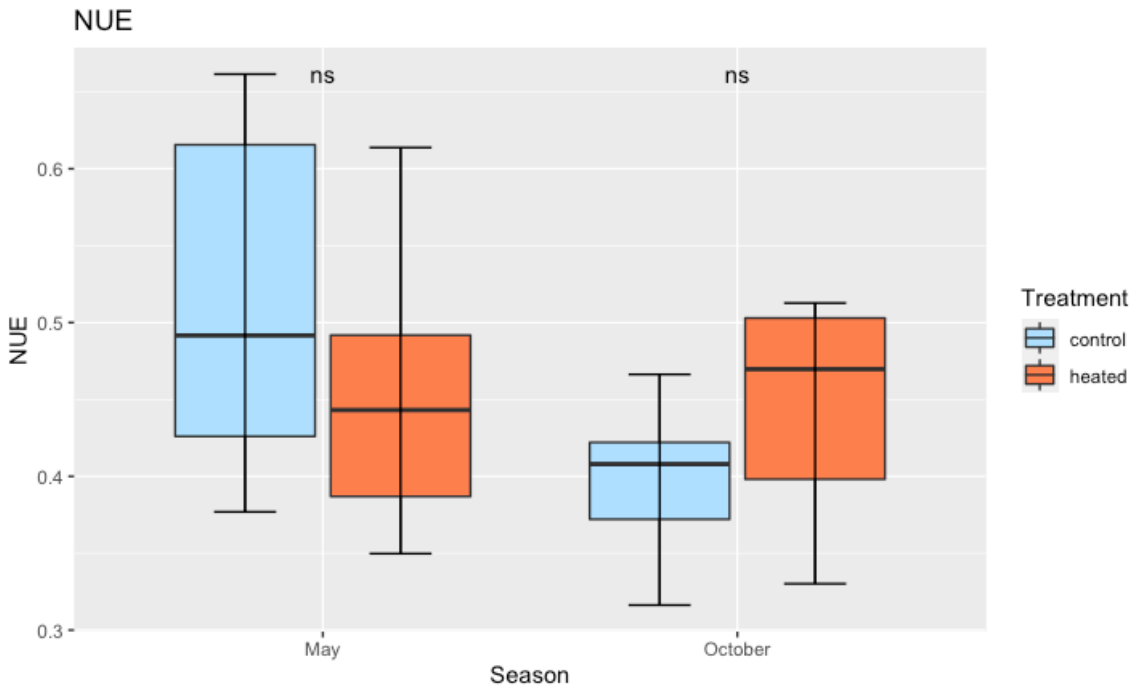


Fig. S2: Nitrogen use efficiency in May and October.

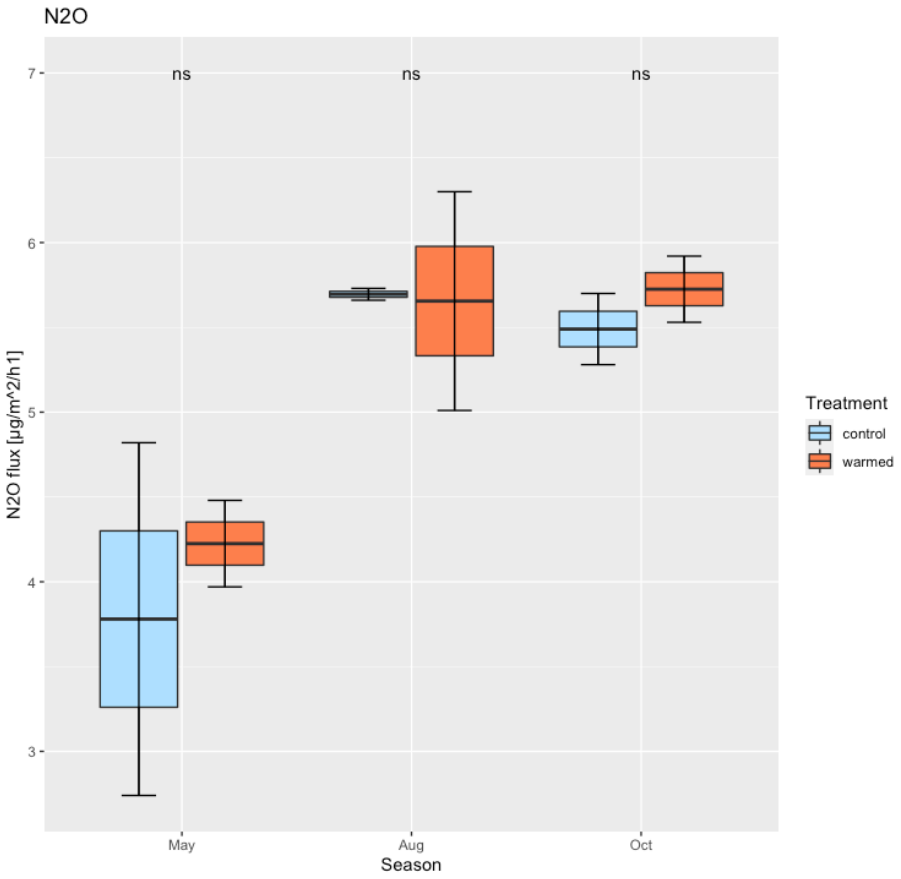


Fig. S3: N_2O fluxes in warmed and control plots for May, August and October. Data from Heinzle J. & A. Schindlbacher.

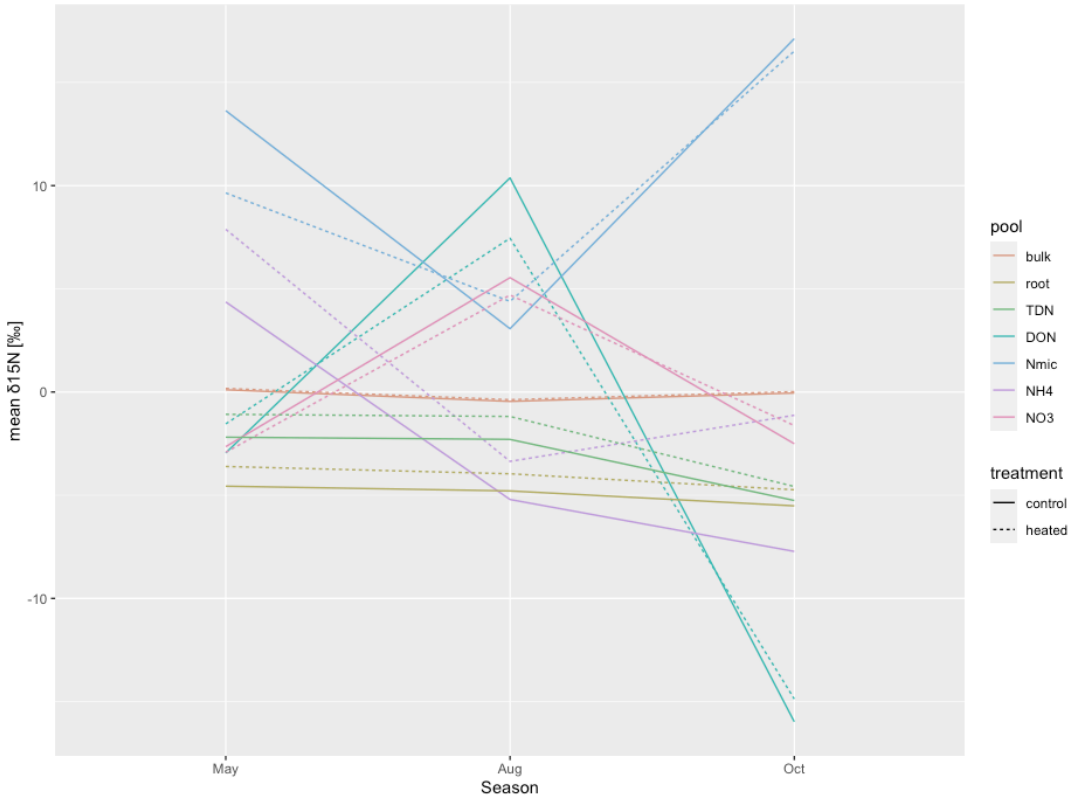


Fig. S4: Seasonal changes of mean $\delta^{15}N$ values for soil nitrogen in comparison with other nitrogen pools. Roots and NH_4^+ were significantly enriched with ^{15}N in warmed plots.

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