Publizierbarer Endbericht

Gilt für Studien aus der Programmlinie Forschung

A) Projektdaten

Allgemeines zum Projekt	
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Langtitel:	Functional response of forest ecosystems to N deposition and climate change
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Programm inkl. Jahr:	ACRP 7th Call (2014)
Dauer:	
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Projekt- und KooperationspartnerIn (inkl. Bundesland):	Umweltbundesamt GmbH (Wien)
Schlagwörter:	Nitrogen deposition, carbon sequestration, decomposition
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Klimafonds-Nr:	B464719
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B) Projektübersicht

1 Kurzfassung

Der größte Anteil an organischem Kohlenstoff ist in Böden – vor allem in Waldböden – in Form von organischer Substanz gespeichert. Dieser Kohlenstoffspeicher enthält doppelt so viel Kohlenstoff, wie in allen Pflanzen und der Atmosphäre gemeinsam vorzufinden ist. Natürliche CO_2-Ströme aus Böden zurück in die pflanzliche Biosphäre sind zehnmal höher als industrielle oder künstliche Ströme, so dass jeder größere physikalische, biologische oder anthropogene Prozess, der die Umwandlungsrate der organischen Substanz des Bodens in atmosphärisches CO_2 (Zersetzung) ändert, tiefgreifende Auswirkungen auf den globalen Kohlenstoffhaushalt und damit auf den Klimawandel haben wird. Durch eine erhöhte Mineralisierung aufgrund eines Temperaturanstieges, ist in Waldböden mit einer höheren Konzentration von anorganische Stickstoff zu rechnen. Außerdem zählt die erhöhte reaktive Stickstoff-Deposition aus der Atmosphäre zu den unmittelbaren Triebkräften des globalen Klimawandels. Diese beiden Faktoren bedrohen eine der wichtigsten Ökosystemdienstleistungen des Bodens - die Kohlenstoff-Sequestrierung.

Wir haben die Auswirkungen eines simulierten atmosphärischen Stickstoffeintrages auf die Kohlenstoffbindung im Waldboden gemessen und mit einem experimentellen Ansatz die Folgen für die Ökosystemfunktionen untersucht. Da eins plus eins in der Biologie selten gleich zwei ist, haben wir eine neuartige Toolbox aus Stabil Isotopen- und Molekulartechniken verwendet, um die isotopischen und molekularen Wege durch die mikrobielle Biomasse zu verfolgen und die zeitgleich im Feld stattfindenden biogeochemischen Prozesse zu messen. Auf diese Weise haben wir die Folgen anthropogener Einflüsse vor dem Hintergrund des Klimawandels in österreichischen und europäischen Wäldern untersucht und somit zu einem wachsenden Wissensstand beigetragen. Über zwei Jahre hinweg haben wir entlang eines europäischen Klimagradienten, in räumlich und zeitlich replizierten Experimenten, den Abbau von markiertem Streu im Wald verfolgt.

Im Gegensatz zu der ursprünglich gestellten Hypothese, jedoch im Einklang mit dem sich abzeichnenden Konsens, der sich aus der aktuellen Literatur ergibt, fanden wir keine Hinweise auf eine beschleunigte Zersetzung unter der +N-Behandlung. Detailliertere Prozessstudien ergaben, dass der Abbau organischer Substanzen im Boden durch +N-Behandlungen verlangsamt und die Bodenatmung reduziert wurde. Darüber hinaus beobachteten wir minimale Auswirkungen der +N-Behandlung auf die mikrobielle Populationsstruktur oder -funktion, die durch Phospholipid-Fettsäureanalysen und moderner molekularer Hochdurchsatz-Sequenzierungsverfahren bestimmt wurden. Schließlich wurden Beobachtungs- und Versuchsdatensätze aus dem Plotmaßstab hochskaliert und in das vollständig gekoppelte Kohlenstoff-Stickstoff-Modell Landscape DNDC zur Kalibrierung und Validierung eingespeist.

Eine umsichtige Politik hat dafür gesorgt, dass die Stickstoff-Emissionen weiter abnehmen und von 1990 bis 2015 wurde ein Rückgang von 50% bei NO_x und 30% bei NH_3 beobachtet, aber bis heute spiegelt sich dieser Rückgang nicht in den Depositionsraten wider und hat das Risiko einer Eutrophierung in weiten Teilen Europas nicht beseitigt. Glücklicherweise deuten unsere Studien darauf hin, dass diese Stickstoff-Vermächtnisse

keine Auswirkungen auf die Stimulierung des Kohlenstoffabbaus im Boden und auf Treibhausgasverluste in Wäldern haben werden. Jedoch legen unsere Studien nahe, dass die Stickstoff-Belastung erhebliche Auswirkungen auf die natürlichen Nährstoffkreisläufe in Wäldern hat, was Auswirkungen auf die Gesundheit und Nährstoffversorgung der Wälder haben könnte.

2 Executive Summary

(max. 2 Seiten, Sprache Englisch)
Siehe oben.

Soils, particularly forest soils, contain the largest organic carbon pool, containing twice as much C than is in all the earth's plants and atmosphere combined in the form of soil organic matter. Natural fluxes of CO_2 from soils back to the plant based biosphere are ten times higher than industrial or manmade fluxes, thus any major physical, biological or anthropogenic process that alters the rate of conversion of soil organic matter to atmospheric CO_2 (decomposition), will have profound implications for the global C budget and consequently climate change. Forest soil in-organic-N concentrations are predicted to increase as the result of increased mineralization due to temperature increases, moreover elevated atmospheric reactive N deposition is considered one the proximate drivers of global climate change, together threatening one of the soil's key ecosystem services, C sequestration.

We measured the impact of simulated atmospheric nitrogen deposition, on forest soil carbon sequestration and examined the consequences for ecosystem function using an experimental approach. We traced the isotopic and molecular pathways through the microbial biomass and measured the concurrent biogeochemical processes as they happened in the field, using a novel tool box of stable isotope and molecular techniques, as one plus one rarely equals two in biology, in doing so we contributed to a growing body of knowledge; studying the consequences of anthropogenic inputs against a background of climate change in Austrian and European forests.

We directly traced the decomposition of labelled litter in the forest, over two years across a European climatic gradient in highly spatially and temporally replicated experiments. Contrary to the initially posited hypothesis, we found no evidence of accelerated decomposition under the +N treatment in line with the emerging consensus evident from the current literature. Moreover, in-depth process studies revealed that soil organic matter decomposition was decelerated under +N treatments and soil respiration reduced. Furthermore, we observed minimal impacts of +N treatment on the microbial community structure or function as determined by phospholipid fatty acid analysis and state of the art high-throughput molecular sequencing.

Finally, observational and experimental data sets from the plot scale were scaled up and fed into the fully coupled carbon-nitrogen model Landscape DNDC for calibration and validation.

Prudent policy has ensured that N-emissions continue to decrease, with a 50% drop in NO_x and 30% declines in NH_3, observed from 1990 up until 2015, although to date, this decline is not reflected in deposition rates and has not removed the risk of eutrophication in major parts of Europe. Fortunately, what our studies suggest is that these N-pollution legacies, will not have knock-on effects in forests, in terms of on stimulating soil carbon

decomposition and greenhouse gas losses. However, what they do suggest is that N-pollution has major effects on the natural nutrient cycles in the forest which might have implications for forest health and nutrition.

3 Hintergrund und Zielsetzung

(max. 2 Seiten) Beschreibung von Ausgangslage, Aufgabenstellung und Zielsetzung.

Forest ecosystems store vast amounts of carbon (C) typically 0.5->2 Mg C ha^{-1} a^{-1} in the form of standing biomass and in temperate systems in their soils; Hence forests are one of the main components of the terrestrial carbon cycle (McKinley et al., 2011).

Elevated atmospheric reactive N deposition is considered one the proximate drivers of global climate change, threatening biodiversity and one of the soil's key ecosystem services, C sequestration. Soils contain the largest organic C pool, containing twice as much C than is in all the earth's plants and atmosphere combined totalling 2400 Pg C when calculated to a soil depth of 2m, to put that in perspective it is 240 times the annual fossil fuel emission (~10Pg). Soil organic carbon is in dominantly in the form of soil organic matter (SOM) (Houghton 2007), any major physical, biological or anthropogenic process that alters the rate of conversion of soil organic matter to atmospheric CO_2 (decomposition), will have profound implications for the global C budget and consequently climate change.

Forest soil inorganic-N concentrations are predicted to increase as the result of increased mineralization due to temperature increases (Schütt et al., 2014, Nadelhofer et al., 1991). At the global scale N deposition rates are predicted to double by 2050 (Galloway et al., 2004) and have significant consequences for above-ground plant diversity especially in low N ecosystems such as forests (Bobbink et al., 2010, Bobbink et al., 1998).

However, experimental results on the effects of increased N input on SOM decomposition are inconsistent, reporting positive, negative and neutral responses of SOM to N input ($^{+}$Conde et al., 2005; $^{+}$de Graaff et al., 2006,$^{-}$Janssens et al., 2010^{neutral} Liljeroth et al., 1994). This proximate N mediated mechanism of decelerated or accelerated SOM breakdown could enhance soil carbon storage or push climate change towards a tipping point.

In Europe, although N compounds in precipitation have decreased since the year 1990, with a 50% drop in NOx and 30% decline in NH_3 up until 2015 (Dirnböck et al., 2018, Tørseth et al., 2012), future reductions are not expected to remove the risk of eutrophication (Posch et al., 2012, Dirnböck 2018). Moreover, in a forestry context impacts of increased N deposition and from fertilizer usage have to be studied experimentally and true impacts of N deposition can only be elucidated by conducting medium to long-term studies, that simulate N deposition appropriately and where the same response variables are measured over comparable timescales.

Estimating soil carbon fluxes and soil carbon inventories using process models relies on process understanding, parametrization and adequate input variables, they will therefore have a degree of associated uncertainty. Empirical models tend to statistically evaluate measured values to produce emission factors, with an inherent uncertainty and bias due to scale, representivity and locality of measurement sites. It has been suggested

that process based models are the most applicable for producing robust frameworks for estimating soil carbon stocks and their response to climate change (Paustian et al.,2016).

Prior to the emerging paradigm shift, that stabilized soil organic matter is primarily derived from microbial detritus rather than direct plant origin, meta-data analyses and the model based hypotheses of the time application (2014-2015) stated that "Global soil C storage is controlled by microbial scale processes of fungal competition for available nitrogen (N)" (Averille et al., 2014, Orwin et al., 2011, Schimel and Bennett 2004). They posited from their meta-analysis and a number of impressive correlations, that plant fungal symbiont community structure (particularly ectomycorrhizal and ericoid mycorrhiza (EEM)) exerted a greater fundamental control over soil C storage than temperature, precipitation or net primary production (Averill et al., 2014). This was deemed important as live ectomycorrhizal biomass can contribute up to 15% of the soil organic matter in temperate coniferous forests (Vogt et al 1982). This in turn was seen as highly significant given the sensitivity of fungal community structure to inputs of N (Lilleskov et al., 2011) and the consequences for higher tropic levels. In turn Averill et al., 2014 initially suggested that higher SOM in EEM systems was the result of EEM fungi's ability to uptake and assimilate low molecular weight organic N. Their hypothesis was that EEM's effectively scavenge all available organic and inorganic N leaving little N for the growth of the free-living decomposer microbial community and preventing further breakdown of SOM (Orwin et al., 2011). They suggested that changes in nutrient status could result in a chain reaction of interacting microbial mechanisms which in turn could lead to the shifts in underlying ecosystem biogeochemical process rates. We set out to test these hypothesises and to determine the causation, in a series of laboratory and field experiments to provide detailed process based information

4 Projektinhalt und Ergebnis(se)

(max. 20 Seiten)
Darstellung des Projektes, der Ziele und der im Rahmen des Projektes durchgeführten Aktivitäten. Darstellung der wesentlichen Arbeitspakete und Aktivitäten. Präsentation der Projektergebnisse.

Details of microbe-dependent biogeochemical feedback mechanisms on N and C dynamics in European forest ecosystems are limited and are often based on nitrogen pollution deposition gradients where correlation rather than causality is established. Although much is known about the microbial diversity of these systems (van der Linde et al., 2018) determining the impact of community structure changes on ecosystem processes has been lacking. Given global trends of increasing atmospheric N deposition and the growing potential use of inorganic N fertilizer in forestry, the function of forest soils as a carbon sink is potentially under threat, with major implications for global climate change.

We set out to investigate this hypothesis experimentally.

1) Does N availability control the rate of organic matter breakdown in an Austrian mixed forest?
2) Is the change in SOM breakdown rate related to a shift of the community structure or community activity?
3) Does increased N availability influence the gross N mineralization and nitrification soil/forest ecosystems?

Hypothesis 1: Increased N availability will lead to increased rate of organic matter breakdown in nutrient –poor forest ecosystems through increased N availability in the pool accessible to free-living saprophytic microorganisms.

Hypothesis 2: N enrichment will significantly alter the fungal biomass community structure and function which in turn will increased SOM decomposition.

Hypothesis 3: The "bottom-up" control coupled with increased N mineralization will lead to positive feedbacks in terms of increased inorganic N accumulation.

We endevoured to investigate whether increased N availability affects coniferous forest soil C turnover, over a broad climatic gradient Sweden to the South of France. All sites had established replicated simulated N deposition treatments and have been running since 2010, as part of the trans-European ALTER-net-MSII network (apart from the UK site).

We aimed to:

- quantify the medium-term effects of N fertilization on C decomposition across a broad biogeographic climatic gradient
- investigate impacts of N deposition on underlying biogeochemical processes of carbon decomposition
- determine whether N deposition changes the fungal community structure and its functional capacity
- study the cascade effects and consequences of changing detrital or microbial resource quality
- model Elaborate on the improvement of existing carbon models taking the consequences of different N deposition scenarios on forest soil C.

Summary of Research we proposed.

In **WP1** we will produce a dual labelled forest litter substrate of high ^{15}N enrichment and moderate ^{13}C enrichment. We will generate a water leached labelled substrate, devoid of soluble-available C and N, to focus in on the impacts of EEM mychorrhiza and free living microbial biomass on complex organic N breakdown. The leaching of the substrate will also ensure that the estimates of N and C losses from the in-growth bags are not confounded by leaching of solutes.

This work package was the preliminary base line stable isotope labelled litter production work package, conducted in the laboratory (L1). In this work package two labelled litter types were successfully produced. One of a coniferous species Abies alba (European silver fir or Weiß Tanne) and one of a deciduous species Betula Pendula (silver birch or Hänge-Birke).

In **WP2** a simple trans-European experiment, we will fill nylon mesh growth bags, which allow for fungal entry but not root entry (50µm mesh Pena et al., 2013), with native soil mixed with a defined quantity of isotope substrate and rebury the bags at appropriate depths on the control and N treatment plots at our sites in Austria and across Europe. We will use mass balance and isotope dilution models to calculate the N and C inputs and losses from the bags over the subsequent seasons. This will allow us to trace the C and N into the mychorrizal and free living soil biomass and to determine the C and N relocation associated with OM breakdown and the impacts of nitrogen deposition. We will also use soils from initial and final sampling of the trans-European sites to calculate total soil C&N inventories.

This work package formed the basis of E1 using a litter of Abies alba, growth and labelling of the trees was minimal. We repeated a similar experiment with shorter time intervals using higher initial application rates with the Betula pendula labelled residues (E2).

In **WP3** we will focus on the Austrian site using the in-growth bags we will follow the fate of both labelled N and C in to the specific microbial functional groups using a combination of ^{13}C and Phylo-trap analysis. We will use the same substrate and bags with a larger 1 mm mesh to study and compare the N and C flows into the microbial population using a combination of bulk organism isotope measurements and ^{13}C fatty acid analysis. **PhyloTrap:**A PhyloTrap for the phylogenetic separation of rRNA from environmental samples should be further developed to improve isotope tracing from nutrients to microbial groups.

We successfully conducted ^{13}C phospholipid fatty acid analysis (PLFA) of samples from the E1 and E2 experiments, however we observed no difference in the fatty acid profiles or up-take of the labelled material between the two mesh sizes. Soil Microbial Communities: Microbial community analysis of N-deposition and control plots was done via high-throughput sequencing (HTS) on the Illumina MiSeq platform. Protocols for DNA-extraction, library preparation, bioinformatic analyses and refined taxonomic and ecological affiliation of sequencing data have been established as part of the Wood-N-Climate projects and other related research projects (see e.g. Keiblinger et al. 2018 and Unterwurzacher et al. 2018).

In **WP4** we focus on the Austrian site and will measure the impact of N deposition on the biogeochemical process rates of gross mineralization and nitrification using a series of in-situ isotope pool dilution assays and enzymatic activities (Hood et al., 2003, Prommer 2014). These experiments will also yield more in-depth understanding of the mechanisms driving inorganic and low molecular weight organic N microbial uptake.

These data form the basis of (F1). We found significant differences between gross mineralization and nitrification rates measured in the field and in the laboratory. We thus took the decision to focus on measuring gross rates in the field at the specific times in the year, we measured successfully over the three-year period and gathered convincing data that gross mineralization rates are lower in the nitrogen deposition treatment and gross nitrification rates clearly higher.

In **WP5** we will bring both experimentalists and modellers together in a current C model workshop in-order to discuss further development of existing dynamic C-N deposition scenarios on soil C storage models. We will particularly use Landscape DNDC as an example of such models. It is one of the most elaborated dynamic ecosystem model (Cameron et al. 2013). Landscape DNDC (L-DNDC) is a detailed process based model simulating C, N (e.g. biomass production, GHG emissions, nutrient turnover and leaching) and water turnover and fluxes of terrestrial ecosystems (Haas et al. 2012).

The model developers are leading scientists in the field of ecosystem modelling and will contribute to WP5. The target of the WP is a peer review paper on future model directions.

We integrated the long-term LTER data from the target site Zöbelboden with a coupled C-N model in order to estimate predictive errors current models may produce as to the effects of nitrogen deposition scenarios on ecosystem N and C cycling. We used LandscapeDNDC, which is one of the most elaborated dynamic ecosystem model (Haase et al. 2003). LandscapeDNDC is a detailed process based model simulating C, N (e.g. biomass production, GHG emissions, nutrient turnover and leaching) and water turnover and fluxes of terrestrial ecosystems. Apart from plot-scale applications and owing to additional funding (ACRP CentForCSink, EU Horizon Ecopotential) we were able to initialize LandscapeDNDC at the entire National Park area providing results going beyond the original plans.

Results.

Work package 1. Production of stable isotope labelled material

Table 1. Isotope enrichment and elemental concentrations of leached plant material.

Birch 3	24.09.2015	4,38	52,89	265,54	2,07
Old fir 3	24.09.2015	-25,02	55,47	129,86	1,02
New fir 3	24.09.2015	10,03	55,89	325,63	0,98

As we suspected the carbon and nitrogen isotope enrichment in the old growth fir was significantly lower than that in new growth fir. We used new growth fir for the experiments. Carbon isotope enrichments were far lower than our target values. We suspect that the lighting and humidity conditions in the growth chamber and some infestation by white fly during the labelling period may have been the problem and possibly undetected leakages and technicians worries about high labelling contaminating IRMS equipment. However these labelling methods have been significantly refined, as a result of learning experience in this new laboratory and are currently being used in ACRP Klima Energie Fonds project, Climagrocycle and FWF project Garden soil.

M.M.1 production of appropriately labelled plant material completed.

Trans-European N deposition experiment

E1. Pine needle decomposition. All soils were signifcantly labelled compared to the background values however we did not achieve the level bulk labelling we had anticipated. This was due to the high background organic N and C values in the soils compared to the addition of the labelled residues.

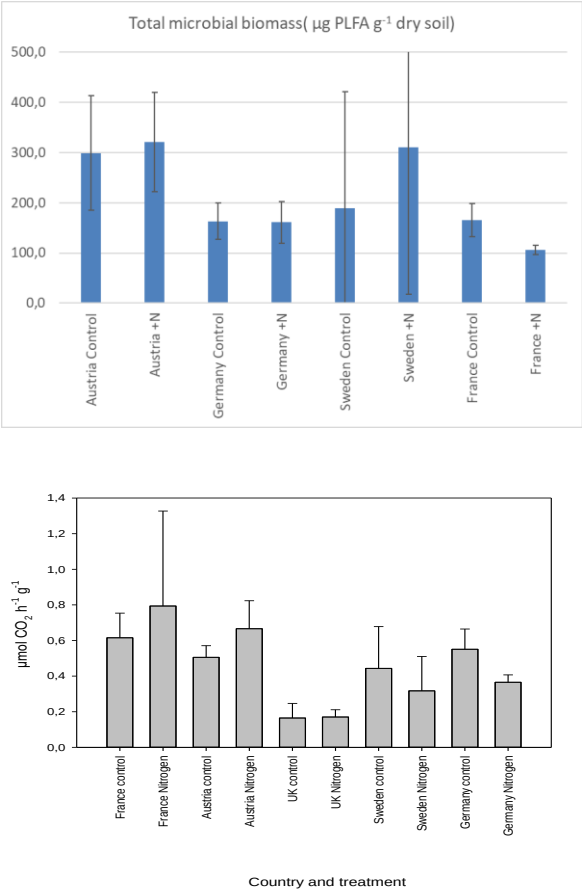
Overall we observed significant country effects with different decompostion rates across countries. In the Austrian soils in the first two samplings we saw no signficant differences in the impact of mesh size on decompostition but we observed signifcant drops in both $\delta^{13}C$ and δ^{15} N over time, δ^{15} N was better fitted with an exponential decay however $\delta^{13}C$ fitted a linear decay. However at the third sampling nearly two years after burial we observed signifcant impact of fertilization and mesh size on bulk soil $\delta^{15}N$ values ($p=0.011$, $p=0.023$) in addition to significant differences in $\delta^{13}C$ ($p<0.001$ for both +N and mesh) suggesting that decomposition was inhibited in the +N treatment, mean residence time of litter N was about 9 years in the +N fine mesh and 4.5 years in all other treatments suggesting that N treatment had the biggest impact on the microbialy mediated decay.

Again in the German soil we saw significant mesh size and +N-treatment effects on bulk soil $\delta^{13}C$ and δ^{15} N values, only after two years ($p_{mesh}=0.009$, $p_{+N}<0.001$, $p_{mesh}=0.032$, $p_{+N}=0.026$ in ^{13}C &^{15}N respectively), again with finer mesh leading to less decomposition. However in the very nitrogen poor Swedish soils only mesh size had a significant effect on bulk soil $\delta^{13}C$ and δ^{15} values again only at the third sampling. In the UK soils only mesh

size had an effect on δ^{15} values at the third sampling. The finer mesh appeared to reduce decomposition consistently in all soils across countries by a factor of around 10% suggesting that the mesofauna play a vital role in the decomposition of the SOM; far more consistently than that of N fertilization. French soil showed no signficant treatment differences after one year however the third sampling was missing as it arrived late.

Microbial biomass carbon as determined by PLFA measurements was not significantly different as a result of N fertilization but significantly different across countries. Bacterial:fungal ratios were not signifcantly different with fertilization in the Austrian soils. However microbial community composition was signifcantly altered in the French and Swedish soils, with prodominant differences in gram negative r strategists, but non in the ectomychorhizal fungi with minimal differences observed in the Austrian and German soils.

Figure 2.1. PLFA analysis total microbial biomass based on carbon content, n=5. T-tests of differences observed in community structure and respiration rates. Autumn 2016 one year after litter bag burial. Error bars +/- standard deviation



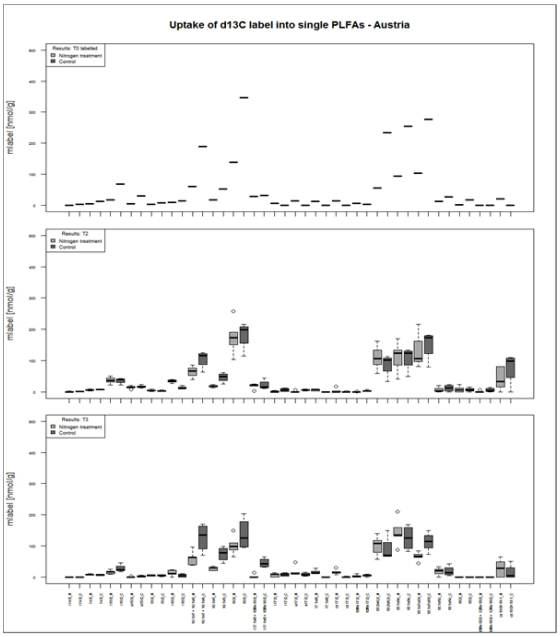
	Austria	Germany	Sweden	France
i14:0	0.80	0.32	0.94	0.52
14:0	0.69	0.54	0.96	0.65
i15:0	0.72	0.15	0.57	0.49
a15:0	0.46	0.85	0.19	0.01
15:0	0.84	0.94	0.01	0.50
i16:0	0.78	0.83	0.10	0.24
16:1w7c	0.54	0.46	0.07	0.00
16:1w6c	0.05	0.25	0.02	0.00
16:1w5c	0.47	0.74	0.00	0.00
16:0	0.82	0.90	0.37	0.01
i17:1w8	0.58	0.47	0.33	0.02
10Me16:0	0.18	0.08	0.13	0.14
i17:0	0.55	0.42	0.73	0.78
a17:0	0.66	0.18	0.12	0.92
17:1w8	0.88	0.23	0.03	0.94
cy17:0	0.48	0.25	0.29	0.76
17:0	0.11	0.59	0.63	0.05
10Me17:0	0.74	0.89	0.08	0.18
18:2w6.9	0.69	0.98	0.17	0.18
18:1w9c	0.41	0.22	0.19	0.13
18:1w7c	0.81	0.93	0.09	0.05
18:1w5c	0.33	0.66	0.00	0.00
18:0	0.47	0.52	0.43	0.05
10Me18:0	0.53	0.48	0.69	0.23
cy19:0a	0.58	0.96	0.25	0.19
cy19:0b	0.85	0.56	0.75	0.03

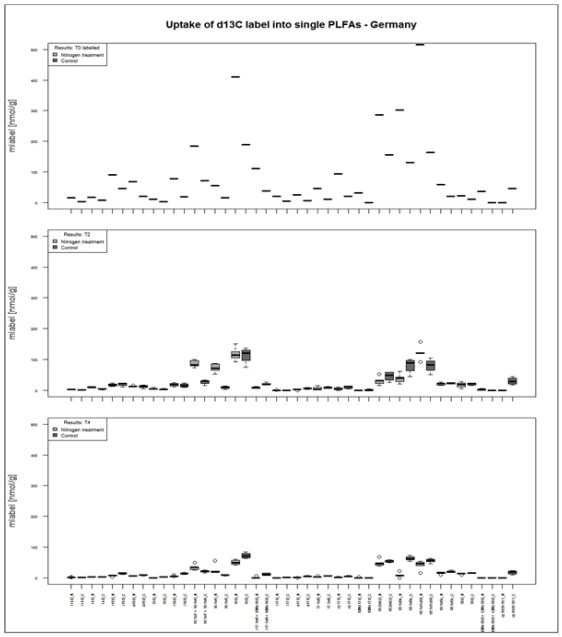
Soil respiration rates were only significantly reduced in the +N in the German soil (p=0.046) although there were significant differences between countries. There were no signifcant differences in between fertilizer treatments of $\delta^{13}C$ of respiration.

EPS: We attempted to develop a new method to determine the isotopic enrichment of the extracellular polysacarides in the soil, the idea being that this is a small active nitrogen and carbon pool where we could observe changes in isotopic enrichments as reflected by microbial activity. Although it the method worked well for nitrogen there was a high and variable carbon blank from the cation exchange resin. We observed significant country differences in soluble organic carbon concentrations, but no treatment differences in the $\delta^{13}C$ of the organic carbon or the EPS. EPS-C amount was not significantly different between treatments or countries but EPS-C in Swedish samples were twice that of German and Austrian samples. We also observed differences in total soluble nitrogen concentrations

between countries but not treatments and saw no significant fertilizer treatment differences in δ^{15} N. We observed signifcant differences between soluble N enrichments and EPS δ^{15} N enrichments.

E2 : Birch litter decomposition. All soils were signifcantly labelled compared to the background values. Again overall we observed significant country effects with different decompostion rates across countries. Based on three-way ANOVA analysis we observed no signifcant differences in labelled litter decomposition rates based on the bulk soil δ^{13}C and δ^{15} values over the four sampling dates as a result of mesh size or fertlization, despite over 600 measurements. We also observed no +N treatment differences in the δ^{13}C of the respired CO_2, but significant country and sampling time influences. We did however see a consistent and highly signifcant depression of respiration as a result of the +N treatment suggesting a major impact on the a key soil function across all the European soils. We observed no significant treatment effects on POx C concentrations across all samples but significant country effects. There was no signifcant impact of mesh on soluble nitrogen concentrations ammonium NH_4^+, nitrate NO_3^- or total free amino acids. There were detectable significant differences in NO_3^- concentrations as a result of the N treatment in the litter bags in the majority of the samplings and all of the countries. There were however no significant differences in NH_4^+ and TFAA concentrations. PLFA analysis (n=5) showed no significant differences community stucture or in uptake of δ^{13}C residue in both German and Austrian soils in Summer (T=2) or later on in the experiment Autumn (T=4).





M2.1. Litter bags distributed and applied, in two experiments.
M2.2-2.4 All bags collected and analysed.

Genomic and functional profiling.

Several approaches for improved efficiency of rRNA capture were tested. Initial recovery was only 6 % of the theoretically available fungal SSU rRNA from a reference samples. Lengthening of the capture oligos could improve recovery to ca. 40 % of the theoretical maximum.

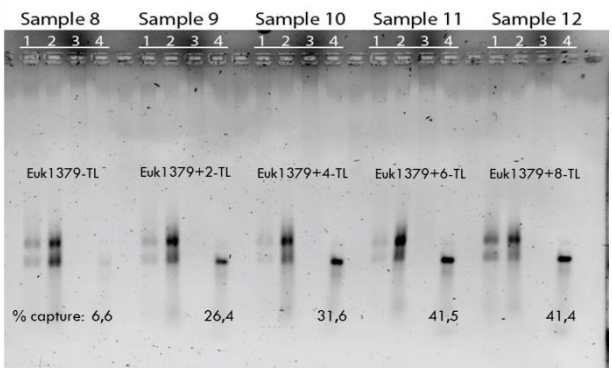


Figure WP3.1: PhyloTrap-capturing of fungal SSU. Original oligo Euk1379 was lengthened by the addition of 2, 4, 6 or 8 nucleotides. Lane 1 shows a dilution of the input RNA, lane 2 depleted rRNA after capture, lane 3 RNA from wash steps and lane 4 the captured RNA after elution from the magnetic beads. The percentage of captured SSU rRNA from the theoretical maximum is indicated below.

Protocols for capturing SSU rRNA were shown to be highly specific for fungi and bacteria, respectively. Additional capturing of LSU rRNA was expected to increase yield roughly by a factor of 3. It was, however, not successful.

Derivatization of RNA according to published protocols (Villas-Bôas et al 2003) to allow GC-separation was tested, but it was concluded that no derivatization occurred. Further protocol testing is therefore necessary.

Microbial Community Analyses

An overview of samples, from which HTS data are available, is given in the table below.

Table WP3.1: Overview of samples with data from high-throughput sequencing community analyses.

Site	Country	Date	# Control Plots	# N-dep. Plots	Horizons	# samples[a]
Zöbelboden	A	Jul 2015	5	5	3	26
Zöbelboden	A	Oct 2015	5	5	3	29
Zöbelboden	A	Aug 2016	5	5	3	19
Zöbelboden	A	Oct 2016	5	5	3	29
Bayreuth	G	2012	2	4	1	6

[a]: Number of samples can deviate from the calculated maximum, as not always all soil horizons were present at selected plots

Bioinformatic analyses have only been finished for the samples Zöbelboden October 2015. For the remaining samples data quality was not sufficient during the first MiSeq run. All steps were therefore repeated, and high-quality data are available in the meantime for further analyses.

The fungal community was dominated by Ascomycota and Basidiomycota. Only a small fraction could be categorized as ectomycorrhizal, which is unusual, but was also found for a nearby site in the Reichraminger Hintergebirge. In October 2015 no treatment specific effects could be observed. The fungal community was remarkably uneven with a few dominant taxa. A similar trend was seen in the Reichraminger Hintergebirge, were abundance of the most dominant taxon increased and diversity decreased from summer to autumn 2015. See Figure WP3.2 for details. Heat and drought in late summer 2015 might have caused the observed phenomenon. Analyses of the remaining data and linkage with additional measurements (soil parameters, enzyme activities etc.) will help to explain the effects.

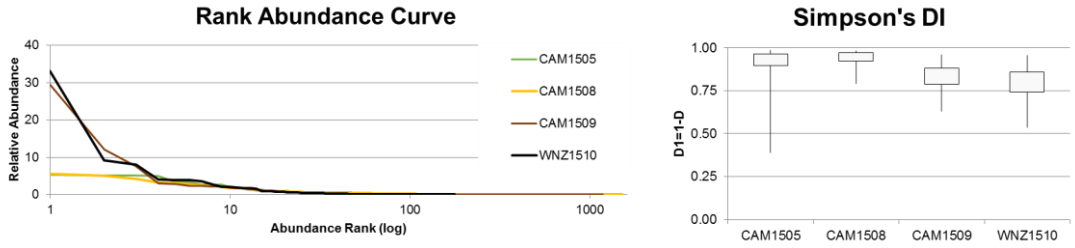


Figure WP3.2: Rank Abundance Curve (left) and Simpson's Diversity Index (1-D) for samples from Zöbelboden October 2015 (WNZ1510) and from reference samples from the Reichraminger Hintergebirge from May, August and September 2015 (CAM1505, CAM1508 and CAM1509).

In T=1 we observed significant differences in both fertilization treatment and country for exoglucanase, ß-Glucosidase, phosphatase and exochitinase, but only country differences for protease activity. Fertilization led to significantly lower enzyme activities. However, at T=3 we observed only significant country differences in exoglucanase, ß-Glucosidase and exochitinase activity, and lower but no significant fertilization effects and no significant differences in urease activity. This is in line the wealth of enzyme data showing similar effects of N depression on enzyme activity.

As we have an significant in-house expertise in ^{13}C PLFA-SIP analysis we substituted the work on mesofauna with a focus on microbial- PLFA uptake of the labelled carbon residue, this is a state of the art method and clearly more publishable. We analysed over 100 samples from experiments in both E1 and E2 using this method. See WP2. For results.

MM3.1 HTP data collected.

MM3.2 SOP's for PLFA and cholesterol extraction established.

MM3.3 PLFA data collected.

Biogeochemical processes

There were signficantly higher carbon and nitrogen stocks in the +N treatment, this was mainly attributable to differenes in the H horizon, where both carbon and nitrogen concentrations were higher. This is phenomena is probably due to the inhibition of fungal activity as a result of high inorganic nitrogen load.

In the laboratory no significant annual differences in laboratory measured respiration rates (µg CO_2 h^{-1}) were observed so data were combined. No treatment differences in respiration, only significant seasonal differences ($p=0.04$) were observed. This difference although not significant could explain the difference in total carbon stocks, results suggests that on average a third more carbon is respired from the control compared to the +N treatment, in line with the litter studies. It shows that, in Autumn rather intuitively, significantly more carbon is respired, possibly a consequence of higher fungal activity, also evident from fungal fruiting behaviour. However care must be taken when interpreting these results as all these tests were run at laboratory temperature of 20°C; temperatures rarely seen in the field in Autumn. Interestingly it suggests that more recalcitrant SOM carbon is respired in the control in Autumn, as in the bulk soil we measured a significant although not particularly predictive negative relationship between % soil carbon and $\delta^{13}C$ ($p<0.001$ $r^2=0.22$, $n=48$). Soils with high SOC had more negative $\delta^{13}C$ values and those with low % soil carbon less negative $\delta^{13}C$ values. The fact that the control treatment respiration was lower suggests control treatment soil is catabolizing this recalcitrant soil organic carbon. However, we saw no significant differences in the A horizon bulk % SOM carbon between treatments, but did observe over all higher carbon stocks in the +N treatment concurrent with these observations.

Table 4.1. Carbon and nitrogen stocks measured in soil.

AT15	mg SOM-C per cm^2 to a depth of 30 cm	To depth 30 cm	mg SOM-N per cm^2 to a depth of 30 cm	To depth 30 cm

	H	A	B	Kg SOM C m^{-2}	t SOM C ha^{-1}	H	A	B	Kg SOM N m^{-2}	t SOM N ha^{-1}
+N	112.3	516.5	527.9	11.6	115.7	4.9	27.3	25.8	0.6	5.8
	73.3	*170.0*	*221.2*	*0.2*	*1.7*	*3.2*	*9.0*	*10.8*	*0.0*	*0.1*
Significance	*0.006*			*0.001*	*0.001*	*0.024*			*0.024*	*0.024*
Control	60.0	467.4	539.2	10.7	106.7	3.0	24.0	30.4	0.6	5.7
	65.3	*212.7*	*298.5*	*0.7*	*6.9*	*3.3*	*10.9*	*16.8*	*0.0*	*0.5*

Table 4.2. Soil weights and POx C stocks measured in soil.

AT15	To depth 30 cm		mg Pox C per cm^2 to a depth of 30 cm			To depth 30 cm
	Kg soil m^{-2}	t soil ha^{-1}	H	A	B	Kg Pox C ha^{-1}
+N	213.8	2122	1.8	6.7	5.6	141.1
	52.9	*408*	*1.2*	*2.2*	*2.3*	*5.2*
Significance						*0.001*
Control	213.1	2175	1.5	8.3	6.4	162.4
	56.9	567	*1.7*	*3.8*	*3.5*	*10.9*

Table 4.3 Laboratory measured respiration rates and isotopic ratios.

Group	Mean µg CO_2 h^{-1}	SEM	Mean δ ^{13}C respiration	SEM	
Cnt X Autumn	21.6	2.8	-27.7	0.3	a
Cnt X Summer	12.0	2.9	-26.7	0.3	b
+N X Autumn	14.9	2.8	-26.8	0.3	b
+N X Summer	13.6	2.6	-27.1	0.3	b

Table 4.4. Respiration rates.

	Field readings		Normalized to 20°C	
	Mean g CO_2 m^2	SEM	Mean g CO_2 m^2	SEM
Summer control	0.43	0.03	0.56	0.05
Summer + N	0.36	0.03	0.49	0.05
Autumn control	0.29	0.04	0.65	0.06
Autumn +N	0.21	0.04	0.52	0.06
Spring control	0.28	0.06	0.37	0.10
Spring + N	0.35	0.06	0.46	0.10

There were no significant differences between treatments or season for raw field readings however when values were normalized to 20°C the approximate temperature at which the laboratory samples were taken we observed significantly higher apparent respiration rates in the Autumn which matched well with the laboratory data.

Table 4.5. Gross process data (+/-standard error) based on orthogonal crosses.

	Gross mineralization		Gross nitrification	
Flux data µg N g dry $soil^{-1}$ day^{-1}	*Control*	*Plus nitrogen*	*Control*	*Plus nitrogen*

S15	Influx	**0.02**	*0.02*	**11.89**	*4.22*	**0.02**	*0.00*	**0.15**	*0.04*
	Efflux	**1.77**	*0.98*	**12.92**	*4.03*	**0.14**	*0.03*	**0.16**	*0.04*
	MRT	18.19		3.85		2.8		2.6	
SU 16	Influx	**66.57**	*15.55*	**31.48**	*10.33*	**1.88**	*0.31*	**3.17**	*0.42*
	Efflux	**83.05**	*18.55*	**4.55**	*2.22*	**6.55**	*0.86*	**8.11**	*0.85*
	MRT	0.40		11.51		0.1		0.2	
AT16	Influx	**51.34**	*8.12*	**2.46**	*0.94*	**5.17**	*0.79*	**3.48**	*0.86*
	Efflux	**71.02**	*10.05*	**8.17**	*2.45*	**8.03**	*1.18*	**70.33**	*30.38*
	MRT	0.49		3.69		0.3		0.1	
SPR17	Influx	**19.50**	*4.45*	**37.39**	*9.84*	**10.52**	*3.99*	**3.68**	*1.15*
	Efflux	**23.33**	*5.33*	**26.07**	*6.61*	**14.42**	*5.28*	**4.09**	*1.76*
	MRT	1.78		2.44		0.4		3.9	
SU17	Influx	**56.71**	*15.42*	**41.28**	*12.38*	**2.48**	*0.82*	**27.29**	*7.11*
	Efflux	**27.61**	*9.16*	**34.05**	*11.23*	**2.77**	*0.83*	**18.76**	*5.01*
	MRT	2.12		1.73		1.3		3.4	
AT17	Influx	**87.93**	*20.87*	**72.92**	*23.51*	**3.65**	*1.43*	**3.38**	*0.63*
	Efflux	**75.13**	*20.98*	**99.59**	*33.24*	**5.35**	*1.42*	**8.59**	*1.44*
	MRT	0.99		1.00		0.3		0.3	

MRT=mean residence time in days. Note only influx tests shown for significance. Colors show N treatment differences as determined by ANOVA. No color=no-significant difference. Green $p<0.05$, orange $p<0.01$, red $p<0.001$. Field measured gross mineralization rates were less than 100 µg N g soil^{-1} per day, generally around 60 µg N g soil^{-1} day^{-1} in the controls and 30 µg N g soil^{-1} day in the N treatments and this depressive effect was significant for the initial sampling dates. N treatment had a significant positive effect on gross nitrification rates.

Model integration and scenario analysis. Modelled tree stem biomass and soil respiration was well in line with observations at the two intensively studied plots IP1 and IP2. Stem biomass development between 1996 and 2010 (IP1) and between 1998 and 2016 (IP2) of the two dominant tree species could be modelled with a Pearson $r > 0.99$ and Kling-Gupta efficiency between 0.57 and 0.97. Daily soil respiration was modelled with a Pearson $r = 0.97$ and Kling-Gupta efficiency of 0.93 at IP1, whereas at IP2 Pearson r was 0.66 and Kling-Gupta efficiency was 0.36 (Figure WP5.1). At IP1, the model underestimated soil respiration with a mean error of 4.3% (IP1), and overestimated soil respiration with a mean error of 15.2% at IP2. Modelled seasonal peak ground vegetation biomass, which is an important driver of C-N processes in these forests, corresponded well with measured biomass records at IP1 and IP2 showing deviations between 0.01 and 0.05 t ha^{-1}, representing 1.3% and 8.3%, respectively.

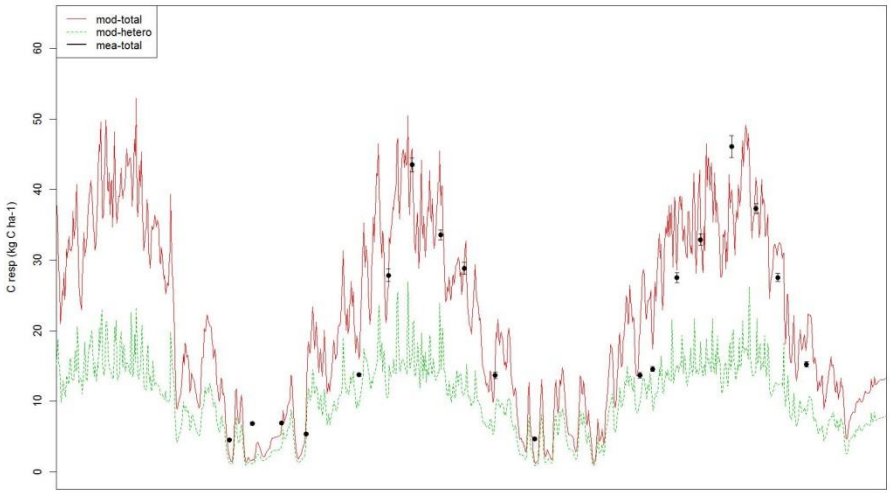


Figure WP5.1: Observed mean (±SD) versus modelled daily soil respiration at intensive plot 1 (LTER Zöbelboden, IP1) for three years. Red line: autotrophic respiration; green line: heterotrophic respiration.

A plot-scale application of the model was used to reveal drought effects on nitrate leaching. We found that in the year after a summer drought, nitrate export via seepage water was significantly elevated compared to the long-term mean. While in normal years, nitrate export was primarily controlled by seepage water volume, after a summer drought these controls changed and nitrate export was determined by nitrate input via precipitation, tree N uptake, and vapor-pressure deficit. This work has been submitted to the journal Environmental Pollution but was rejected. A revised version will be submitted to another journal soon.

Regarding landscape scale modelling, we paid particular attention to quantify the effect of woody and non-woody understory vegetation on net ecosystem production (NEP) because the often open tree canopies in mountain forests increase the importance of understory for C and N cycling. We could show that in the National Park area was a C sink between 2.4±1.7 t C $ha^{-1}y^{-1}$ and 3.2±1.4 t C $ha^{-1}y^{-1}$ (between 2000 and 2014, Figure WP5.2). Furthermore, woody and non-woody understory vegetation caused between 16 and 37% higher regional NEP as compared to a bare soil scenario over a 15-year period. The mean annual contribution of the understory to NEP was in the same order of magnitude as the average annual European (EU-25) forest C sink. This work has been submitted to the journal Landscape Ecology including a discussion and conclusions about potential model improvements.

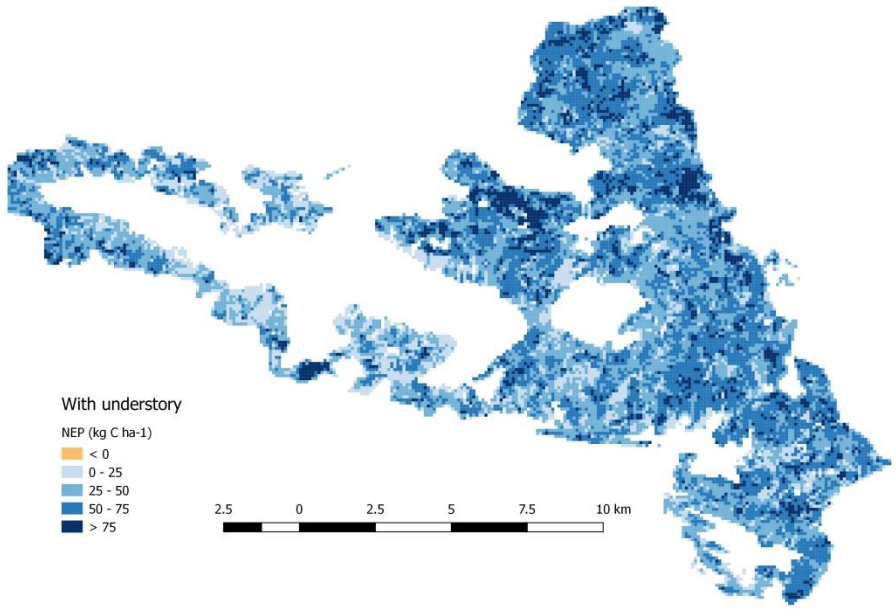


Figure WP5.2: Cumulative net C sink for the National Park Kalkalapen from 2000 to 2014 as modelled with LandscapeDNDC.

In addition, the conclusions from the empirical and modelling efforts carried out within Wood-N-Climate were taken up in two recently published reviews about N effects in ecosystems (Schmitz et al. 2019, Perring et al. 2018).

5 Schlussfolgerungen und Empfehlungen

(max. 5 Seiten)

Beschreibung der wesentlichen Projektergebnisse. Welche Schlussfolgerungen können daraus abgeleitet werden, welche Empfehlungen können gegeben werden?

We measured the impact of simulated atmospheric nitrogen deposition, on forest soil carbon sequestration and examined the consequences for ecosystem function using an experimental approach. We directly traced the decomposition of labelled litter in the forest, over two years across a European climatic gradient in highly spatially and temporally replicated experiments and showed that the addition of inorganic N as a proxy for reactive nitrogen pollution did not accelerate decomposition rates, contrary to our initial hypothesis. "1. Increased N availability will lead to increased rates of organic matter breakdown in nutrient-poor forest ecosystems through increased N availability in the pool accessible to free living microorganisms", that was based on observations and correlations from Averille et al., in 2014, who suggested that higher SOM in EEM systems were the result of fungi's ability to uptake low weight molecular organic N and scavenge it all, leaving little for the free living decomposer community. It has become clearer over the last few years that the majority of long term carbon storage in soils, particularly in forest soils is derived from fungal biomass and this provides the alternate explanation for the initial hypothesis posited by Averille et al., (2014). Our experimental work also confirms this initial hypothesis was possibly flawed. We tackled the hypothesis from a number of perspectives, using novel in depth and state of the art methods, in the field, what our studies have shown is that is it possible to undertake such studies in the field that give sensible relevant data that capture the important processes for feeding into global models and the general discourse on organic matter turnover. It should be stressed that to date most of the studies of this isotopic and molecular nature have been conducted in short term experiments in the laboratory and then extrapolated back to the field. Our new knowledge about the role of

mychorrizal association and the role it plays in tree nutrition and nitrogen uptake and turnover in soils, renders many of the laboratory studies questionable.

In contrast to hypothesis 2, we found no molecular or convincing evidence from the PLFA data that N enrichment in Austria alters the fungal biomass community structure (and function) which in turn we hypothesized would increase SOM decomposition. On the contrary in Austria we found significant negative impacts of N enrichment on community function, evident from reduced rates of SOM decomposition based on the bulk data, litter bag experiments, respiration measurement and detailed process data. Lack of response on the microbial structure might have been due to the fact that these Austrian sites are considered N saturated and that our controls are so altered by legacy N pollution that we see no effect of the nitrogen treatments. Similarly, we saw no structural differences in Germany and contrasting functional responses. We did see observe some significant effects on the microbial community structure in the PLFA data in both Sweden and France but despite these differences we saw no impact in terms of accelerated decomposition as a result of N addition, rather decelerated decomposition. We conclude that these forest soils which have an extremely high C:N ratio, are not sensitive to changes in the stoichiometry per se, but to deactivation of the fungal activity.

We saw no evidence to support the third hypothesis "bottom up control coupled with increased mineralization will lead to positive feedbacks in inorganic N accumulation".

However, what we clearly showed was the role of mesofauna in forest soil litter decomposition, around 10%, differences and again show the importance of field based studies, as low storage temperatures in laboratory studies will kill or inactivate faunal activity.

We showed that over a broad climatic gradient N fertilization leads to a neutral impact or decrease in C decomposition in soils.

The biogeochemistry suggested that fungal activity rather than ecosystem stoichiometry is a stronger driver of decomposition and carbon storage, and as such is sensitive to reactive N fertilization/pollution.

We have shown that these labelled litter bag experiments provide excellent data for understanding the processes in nature across large geographic scales. Their implementation was simple and effective, once we had ironed out a few teething difficulties. The reproducibility was astounding in many cases given the heterogeneity of the field sites. These stable-isotope based experimental systems, do provide the essential gold standard data on which to build SOM models and make future predictions on the response of ecosystems to climate changes.

The labelling bag set-up means that extensive, highly replicated studies can be undertaken at reasonable cost, which can run long term with realistic resources.

Based on the findings of the study, although it appears we need not worry about the impact of reactive nitrogen destroying the carbon stocks of forest soils and creating a tipping point in the carbon sequestration system; we should however be concerned that there seems to be major effects of reactive nitrogen on forest fungal activity. This disturbance of the natural forest cycle has implications for Austrian foresters as the fungal activity has been shown to be inextricably linked to forest health and nutrition. Reduction of transport based fossil fuel emissions will go a long way to reducing the reactive nitrogen input into these systems. The peripheral benefits of a transition to low carbon economies should help restore forest health across Austria. It is only with well instrumented, staffed and serviced

monitoring facilities are the true benefits of such clean air policies decisions clear and accountable.

Austrian forest soils have a vast capacity and potential to store carbon, if well managed we could increase that capacity substantially whilst reaping the benefits of the other ecosystem services they provide. This work was conducted on one forest in Austria ideally it would be prudent to study a range of sites across Austria and Europe and to determine the parameters promoting carbon sequestration.

The work highlighted that it is possible to directly measure organic matter turnover in forest soils in realistic time frames, with forest litters, in the field using the novel isotope techniques described herein. These sensitive measurements are essential to underpin the assumption made in the models and can only be executed where the true of complexity of the ecosystem is captured in the experimental design. These methods allow us to do that and further test the hypotheses and drivers of organic matter decomposition in both forest and agricultural systems and to rule out the potential threats to one of our most important stores of carbon globally- the soil!

It has been recently highlighted by leading lights in the scientific community that the soil could save us from the impending disaster of climate change. See www.4p1000.org for further insights. We know soils have the capacity to store enormous amounts of carbon, we know how, and it could be done now! We need to take this opportunity of to leverage this soil centric-stop-gap measure in these times of transitioning to a low carbon economy. Further research to underpin these soil based negative emission strategies is required to ensure the outcomes of recommendations are based on sound and locally appropriate scientific assumptions. With Austrian foresters and the high number of organic farmers there is phenomenal potential to offset our carbon emissions in Austria, and follow the leaders in becoming a carbon neutral country.

C) Projektdetails

6 Methodik

Materials and methods. Production of stable isotope labelled material

In the spring 2015 we collected 5 year old *Abies alba* 30-50 cm height seedlings. Trees were pruned of the lower branches. Branches were tagged to identify point of new growth During the summer months we repeatedly isotopically labelled them with $^{13}CO_2$ and ^{15}N fertilizer using the bespoke isotope labelling chamber. See details below. We produced two residues types new and old growth, as it was suspected that the old growth would not take up the carbon label. From the new material we produced a leached new growth litter material with 10.35 ‰ ^{13}C labelling and 325 ‰ ^{15}N labelling. This was sufficient for the in-growth bag experiments. This material was distributed to our European Partners for incubation in the field experiment E1.

We also collected 2 year old, *Bettula Pendula* seedlings and during the summer months we also repeatedly isotopically labelled them with $^{13}CO_2$ and ^{15}N fertilizer using the bespoke isotope labelling chamber. We produced a leached new growth litter material with 4.38 ‰ ^{13}C labelling and 266 ‰ ^{15}N labelling. This was sufficient for the in-growth bag experiments. This material was distributed to our European Partners for incubation in the field E2.

All trees were re-potted 8.5 by 30 cm grey plastic tubes, with a loamy sand Tulln Soil, to each pot we added air dried soil 777,5 g per pot and covered the soil in 33,82 g of plastic beads to prevent evaporative losses in the chamber.

Carbon and nitrogen labelling method

Labelling was achieved by pulse labelling of the be-spoke Perspex chamber with the dimensions of 70 by 70 by 80 cm. We leak tested the chamber prior to labelling using a methane pulse and measured loss of methane over time by adding a pulse of pure methane allowing a few minutes for mixing then extracting 1 mL samples from the chamber and measuring methane concentrations every ten minutes using a gas chromatograph fitted with a flame ionisation detector. No significant losses of methane were detected over a four-hour period.

Sodium bicarbonate, $NaHCO_3$, Molar mass: 84.007 g/mol, IUPAC ID: Sodium hydrogen carbonate 372382. Sigma Aldrich 98 atom % $NaHCO_3$ was used, in the original Hood et al., (2004) method we used 10 mL of 0.5 molar solution per injection. 84/2 = 41 g $NaHCO_3$ per litre, 4.1 g per 100 mL or 1.25 g per 25 ml. We used the 25 mL of 98% labelled and 225 mL of unlabelled solution to get 10.88 atom % solution. We used 10 mL of labelled (10.88%) solution the flushed the label into the lactic acid inside the chamber with two subsequent injection of 10 mL of un-labelled solution one and two hours after the initial labelling event (this ensured that the most of the expensive label was taken up by the plant and not lost into the atmosphere). CO_2 in the chamber was monitored with a conventional infra-red gas analyser. Labelling was carried out weekly for each tree species.

At each labelling event we filled the inner vessel with 30 mL of 7.5 molar lactic acid. This was made with deionised distilled water with 11.3 M commercially available 85% Lactic acid. Thus we made up a solution of 663 mL in I litre or 66.3 mL in 100 ml.

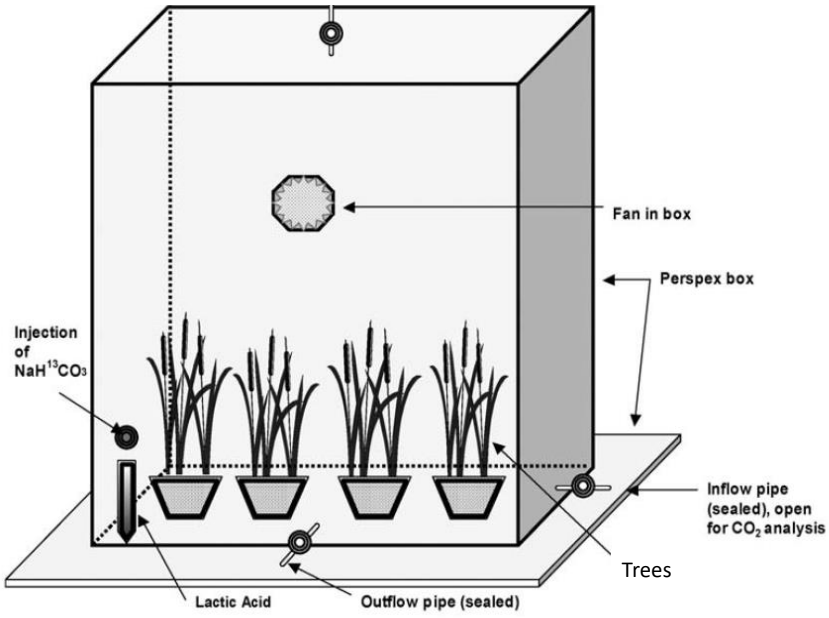


Figure 1.1. Set up of dual labelling chamber.

The N labelling solution was prepared from 10 atom % ^{15}N solution ammonium nitrate, NH_4NO_3. Molar mass: 80.052 g/mol. We applied and equivalent fertilizer rate of 30 kg Nha^{-1} =30/2000000*1000*1000. 1 hectare of soil is 2000000 kg about =30/2 =15 mg per Kg or 15 µg per g 80.052/28*15= 42.8 mg per Kg. If we take 10 mL per pot (we water with it 10 times) we make up 42.8 mg in 100 ml. 20 trees ten times labelling. 20 *100 =2000 42.8*20= 857.76 mg. 0.857 g $(NH_4)(NO_3)$in 2 litres.

Trees were harvested at the on the 15th of September 2015, samples were separated into leaf, stem and root fractions weighed, and coarsely ground. Leaf samples were subsequently repeatedly (twice) leached with boiling water, and re-dried at 40°C to reduce the soluble N and C fractions, in order to assure even labelling and reduce the risk of leaching losses compounding the results. Sub samples of leached materials were dried and finely ground for subsequent isotope analysis.

Samples were analysed for isotope composition at the FAO/IAEA laboratory Seibersdorf, as at the time there was not the available equipment for the analysis at AIT. % N and C as well as ^{15}N and ^{13}C and were determined by a Dumas combustion method with an elemental analyser (Carlo Erba, Italy) linked to an Optima IRMS (Micromass, UK).

Trans-European N deposition experiment

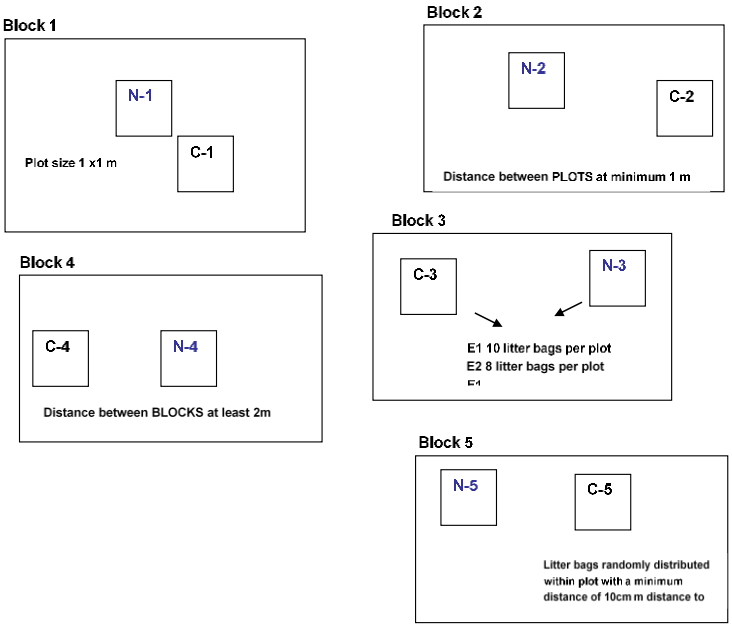
Experimental design, within each country the experiment was carried out in a random block design, each block with each blocks repeated 5 times within the designated site. Blocks were randomly distributed among all suitable substrates within an area of about 20 x 20 m, but with at least 2m between blocks.

Fertilized plots were watered (sprayed) with tap water, containing N as treatment. Unfertilized plots were watered with tap-water only. Each plot received 1 L of the solution. every 3rd week. Fertilization was not conducted during heavy rains but drizzling was accepted if the rainy period lasted several days. Climatic data were noted on the day before and day after fertilization. In all countries apart from the UK the N treatments were started in June 2010, in the UK the treatments were started in 2014.

N-fertilization : According to literature we simulated a nitrogen input of 80 to 100 kg N/ha/yr (equivalent to 8 to 10 g N/m²/yr). This simulates a quite high deposition but ensures a strong gradient to the controlAmount of added N-fertilizer per spraying action is given in the table below.

Table 2.1. Fertilization scheme for +N treatment plots, (NH_4NO_3).

Date of NH_4NO_3 fertilization	Amount of sprayed solution per 1 plot	Amount NH_4NO_3 per 1 plot	Total N/m²/ spraying date	Amount NH_4NO_3 per 5 replicate plots
June 14th	1 liter	2.86 g	1 g N m²	14.3 g
June 21	1 liter	2.86 g	2 g N m²	14.3 g
June 28	1 liter	2.86 g	3 g N m²	14.3 g
July 19	1 liter	2.86 g	4 g N m²	14.3 g
August 9	1 liter	2.86 g	5 g N m²	14.3 g
August 30	1 liter	2.86 g	6 g N m²	14.3 g
September 20	1 liter	2.86 g	7 g N m²	14.3 g
October 11	1 liter	2.86 g	8 g N m²	14.3 g
November 1	1 liter	2.86 g	9 g N m²	14.3 g
November 22	1 liter	2.86 g	10 g N m²	14.3 g



Litter bag experiment E2. Pine litter.

Each of the five participating ALTER-NET site managers in France, Sweden, UK and Germany received pre weighed tubes isotope labelled leached new fir litter (described above) and mixed it with their respective soils (100g), from the respective control or + N site. The mixed soils were then filled into the two types of mesh bags (fine mesh 50 ± 10 micron porosity, this mesh size is fine enough to allow access by bacteria, fungal hyphae, most nematodes and protozoa while restricting access by mesofauna and macrofauna. and coarse mesh, 1000 micro porosity, allows the meso- and macrofauna to enter the bags.

These mesh bags will be placed at the top of the A horizon (mineral layer with some humus). Four bags of each type per replicate, were placed on both treatments. We be buried the samples and marked with a flag in Autumn 2015. Time zero samples and soils were collected and dispatched for analysis.

At the subsequent sampling times the bags were again simultaneously collected from each site and dispatched by post for analysis. On arrival bags were immediately stored at 4°C.

Climatic conditions at the sites have been quantified by three well-established indices: (i) the continentality index CS; (ii) the aridity index AM (; and (iii) a classification of seasonality of precipitation (Bernhardt-Römermann et al.,2011) and additional meteorological data was also collected.

Sampling regime of the E1 bags.

12.10.2015	T=0	
13.05.2016	T=1	214
08.10.2016	T=2	362
31.05.2017	T=3	597 days after burial.

Total C and N analysis and bulk ^{13}C and ^{15}N isotope analysis, elemental isotope ratio mass spectrometry. All samples were dried (40°C for 72 h), finely ground and accurately weighed (3-5 mg) into 9 by 5 mm tin cups. Samples were analyzed using a Thermo Flash 2000 Organic Elemental analyzer, linked to an Thermo Delta V Advantage automated isotope ratio mass spectrometer (IRMS) (Thermo, Bremen, DE). A full complement of internal and external standards was run with the samples in order to calculate isotopic ratios and % C and %N values. The isotope ratios were expressed as parts per thousand

per mille (‰) or d deviation from the internationally recognized standards Vienna Pee Dee Belemnite (VPDB) and ambient air.

Samples for PLFA analysis were prepared using the standard procedure (Watzinger and Hood-Nowonty 2019, see appendix), briefly 1.5 g of soil was extracted in a chloroform-methanol solution, the lipid fraction retained and the phospholipid fraction chromatographically separated from the bulk lipids, methylation was achieved with an alkaline methylation procedure and samples re-solubilized in iso-octane. 13:0 and 19:0 standards and blanks were run with all runs. Samples were run on a gas chromatograph flame ionization detector.

EPS analysis: EPS extraction buffer was prepared in 18 M Ohm H_2O to: 2 mM $Na_3PO_4.12H_2O$ (0.760 g L_1), 4 mM $NaH_2PO_4.H_2O$ (0.552 g L-1),9 mM NaCl (0.526 g L_1), 1 mM KCl (0.0746 g L-1), then adjusted to pH 7 with 1 M HCl and cooled to 4 °C. The extractant for soluble microbial products (SMP) was prepared by adjusting 0.01 M $CaCl_2$ (local rainwater ionic equivalent) to pH 7 with 0.01 M $Ca(OH)_2$, and cooling to 4°C. 1 g fresh soil and 25 mL pre-cooled $CaCl_2$ solution were shaken for 30mins to release soluble microbial products (SMP), then centrifuged at 3200 x g for 30 min, the SMP solution decanted into fresh tubes, frozen stored at -20 °C. EPS was extracted by adding the 5 g of pre weighed cation exchange resin was transferred to the remaining soil sample (centrifuge pellet) together with 25 mL chilled extraction buffer, shaken hard by hand to re-suspend the pellet, and placed on the chilled shaker for a further 2 h. Samples were subsequently centrifuged at 4000 x g for 30 min and the supernatant transferred into new tubes. 2 mL of liquid was dispensed into round bottomed Ependorfs and dried down at 60°C ball milled and 5 mg weighed for isotope analysis.

POM analysis: We extracted particulate organic matter by adding 0.3g of fresh soil and 2 mL of sodium hexamataphosphate solution shaken for 1 hour (50g sodium hexametaphosphate and 7g of anhydrous sodium carbonate in 1 litre). We then filtered, through 53µ mesh as described. We collected the sand and POM fraction on the filter in water and dry down the sample at 60°C. Samples were transferred into a small pre weighed round bottomed Eppendorf dry at 60° C, this POM and fraction was then ground, 5 mg weighed for EA-IRMS C and N and ^{13}C and ^{15}N analysis.

CPOM-C=C_s *W s*SW.

CPOM-C = g C kg soil.

C_s % C POM-sand mix

Ws weight of sand POM mix

SW =dry soil weight initially used.

A conversion factor of 1.724 was used to convert organic matter to organic carbon.

^{13}C of respiration and respiration analysis. 1 g of moist soil was placed in a 20 ml GC vial. An additional 1 ml GC vial containing 0.5 ml of 0.3 M NaOH, was added and date and time noted. This was immediately capped and stored in dark cupboard for one week. Controls-no soil, but NaOH were also set up. After one week the cap was removed and immediately titrated, by removing 400µl of the NaOH into a 10 ml plastic centrifuge tube and adding 400µL of 1 M $BaCl_2$ and vortexed. This was titrated against 0.1M HCL (Titrasol) and phenolphthalein indicator. For isotope analysis the precipitate was filtered out on glass fiber filter papers dried and a subsample of 8 mm diameter circle, punched out of the center of the filter, these were loaded into tin cups and measured on the EA-IRMS with a full range of standards and blanks and reported to VPDB international standards.

Litter bag experiment E2. Birch litter.

In E2 only four of the ALTER-NET sites took part. Austria, Sweden, UK and Germany each received pre weighed tubes isotope labelled leached birch litter (described above, 2.65g of dry leached litter) and mixed it with their respective soils, 240g of a composite sample from all five plots the respective +N treatment or control plots. In the initial experiment we had observed very high variability in SOM contents across the plots, so this overcame some of this error. Five grams of the mixed soils were then filled into the two types of

mesh bags (fine mesh 50 ± 10 micron porosity, this mesh size is fine enough to allow access by bacteria, fungal hyphae, most nematodes and protozoa while restricting access by mesofauna and macrofauna and a coarse mesh, 250 micro porosity, allowing the mesofauna to enter the bags).

These mesh bags were then placed at the top of the A horizon (mineral layer with some humus). Four bags of each type per replicate, were placed on both treatments. In June 2017, time zero samples and soils were collected and dispatched for analysis.

At the subsequent sampling times the bags were again simultaneously collected from each site and dispatched by post for analysis. On arrival bags were immediately stored at 4°C.

01.06.2017	T=0	
14.06.2017	T=1	13 (2 weeks)
28.06.2017	T=2	27 (4 weeks)
26.07.2017	T=3	55 (8weeks)
30.09.2017	T=4	121 days after burial (16 weeks)

On arrival samples a sub-sample of each bag sample was taken for dry weight analysis. All further fresh samples were either then frozen (PLFA) or stored at 4°C and analyzed for:

Total C and N analysis and bulk ^{13} C and ^{15}N isotope analysis, elemental isotope ratio mass spectrometry (see above).

Permanganate oxidizable carbon (POx-C). An aliquot of 0.04 g of soil to 1.0 mL of 0.02 M $KMnO_4$ was added to a 1.5 mL Eppendorf and shaken for 15 minutes on the rotary shaker. Centrifuged at 3000 rpm for 5 minutes 10 µl of the supernatant was pipetted into 990µl of water in an Eppendorf, 200µl of the solution was pipetted into a microtiter plate and read at 550 nm. Standards were included and weight ratios adjusted to fall within standard ranges. 10µl of 0,02 mL in water only $KMnO_4$ was used as the top standard and diluted 1:1 down. (based on Weil et al., 2003) as standard curve.

^{13}C of respiration and respiration analysis. Respiration measurements were made on one-gram fresh soil samples accurately weighed into 12 mL gas tight Labco, Vacuutainers, these were left to stand in the lab at 20°C for one day prior to analysis. All values are reported on a dry weight basis. Samples vials were closed then were repeatedly evacuated and refilled with high purity helium, manually. Samples were automatically injected and measured at 120 and 620 minutes following evacuation, giving a sampling interval of approximately 406 minutes. This was necessary to account for very low background and to get flux measurements. Sampling was done using a CTC analytics CombiPal autosampler injecting 500 µL sample withdrawn from the 12 mL vial held at 20°C into a Thermo-Fisher Trace Gas Chromatograph Ultra. Chromatographic separation was achieved with a He carrier pressure of 60 kPa, via an Agilent PoraPlot Q (25 m x 0.32 mm) starting temperature was 35°C which was held for 3.5 min and heated to 105°C by 40°C min^{-1}. All gases were passed through a high temperature conversion reactor (HTC, maintained at 200°C to prevent sample re-condensation or physical conversion) installed within a ThermoFisher GC-Isolink. Finally, $\delta^{13}C$ of CO_2 was measured by a ThermoFisher DeltaV isotope ratio mass spectrometer detecting the mass-to-charge ratios (m/z) 44, 45 and 46.

Microbial biomass nitrogen and carbon and respective isotope values.

1 g of soil was weighed into a 12 mL vacutainer using at least 3 figure balance, un-fumigated controls (left on bench next to desiccator). Fumigated samples were prepared with 24-hour fumigation in ethanol free chloroform (MTBE stabilized) in an evacuated darkened desiccator, after 24 h, 8mL of 0.5 M K_2SO_4 was added to all samples, shaken 1 hour, centrifuged at 3000 rpm for 10 minutes (Brookes et al., 1985a, 1985b, Amato and Ladd, 1988). Supernatant was extracted using syringe filter set-up. 1 mL of each sample was dried into a round bottomed ependorfs 60°C until dry. Sample was weighed 70-80 mg for carbon and nitrogen analysis for EA-IRMS analysis.

Free amino acids: Were measured in the K_2SO_4 extracts based on (Jones et al., 2002)
The fluorometric method relies on the reaction of free amino acids with o-phthaldialdehyde and 3-mercaptopropionic acid (OPAME) yielding a fluorogenic product. 10 mg OPA is mixed in 1 mL and 20 µL of mercaptopropionic acid added, mixed with 40 mL of potassium tetraborate buffer (0.2 M, pH 9.5). 50 µL aliquots of standards, reagent blanks and K_2SO_4 samples were pipetted into a 96 well microtiter plate with 200 µL OPAME reagent. Samples were read at 340 nm and the emission wavelength set to 450 nm. A full series of glycine standards were prepared and ammonium concentrations were measured with the standard photometric method.

Ammonium and nitrate analysis: Nitrate was determined using the VCl_3/Griess method. This procedure is based on a colorimetric reaction where nitrate is converted to nitrite (NO_2^-) in acidic vanadium chloride (VCl_3) medium. NO_2^- concentration is then measured by direct coupling with the Griess reaction. Absorbance is measured photometrically at 540 nm (Hood-Nowotny, 2010; Miranda, 2001). Ammonium was measured using a modified indophenol method based on the Berthelot reaction. This procedure is a colorimetric method, where ammonium is oxidized to monochloroamine by sodium dichloroisocyanuric acid and subsequently forms a green indophenol compound in the presence of phenolics. Absorbance is measured photometrically at 660 nm (Hood-Nowotny, 2010; Kandeler, 1988).

Samples for ^{13}C PLFA analysis were prepared as above (Watzinger and Hood-Nowonty 2019, see appendix). Samples were run on a gas chromatograph isotope ratio mass spectrometer with a full range of internal and external isotope standards and values reported to VPDB international standards.

Genomic and functional profiling

PhyloTrap

Initial work for phylogenetic separation of rRNA from environmental samples was done before start of the project. Although separation was possible and specific, amounts were not sufficient for standard IRMS. Improvements included optimization of the hybridzation protocols, lengthening of capture oligos and targeting of both, the SSU and the LSU rRNAs. Furthermore, conversion of RNA into formylated ribonucleotide monomers was tested as an alternative for standard IRMS, as GC-IRMS is more sensitive.

Microbial communities

Methods for DNA-extraction, library preparation and bioinformatic analyses followed published procedures, but were refined for the special needs. A protocol for the increase of amplicon diversity during MiSeq runs was developed to improve output of sequencing data. No differences in community data were observed in reference samples with the newly developed technique. Additionally, a separate purification step had to be included for library preparation to account for non-specific byproduct formation during amplification. Manual refinement of automated taxonomic affiliation and ecological categorization greatly contributed to improved quality of the sequencing data and are now routinely implemented in community analyses.

Enzyme analysis: We measured exoglucanase, ß-Glucosidase, exochitinase, phosphatase and urease in duplicate all T=1 and 3 samples. Using modified standard flourometric MUF methods and urea conversion to ammonium.

Measurement of biogeochemical processes

In July 2015 the first field sampling campaign at the Zöblboden site was completed. On this field campaign soils were collected to establish appropriate protocols for field isotope pool dilution labelling these were done in the laboratory.

For carbon and nitrogen inventories soil sampling was conducted on all plots at the Austrian Zöblboden site, ten replicate soil cores down to a depth of 30 cm, from the

top of the H layer, were taken per plot. We chose to take samples based on the layer characteristics, so H layer the organic litter layer, A layer mineral and organic fraction, B layer subsoil. We sampled down to the bed rock which was generally at 30 cm depth using a 1.5 cm diameter auger. On each plot we measured layer depth. Sampling was done in a W formation across the plot. Average bulk density of each layer was also measured, using 200 cm^3 stainless steel rings that were bashed into the soil at the appropriate layer, soil samples were collected in the rings and surfaces carefully cleaned of external debris. These samples were sealed with steel lids on both bottom and top and placed into zip lock bags, these were returned to the lab weighed and subsequently dried in the oven at 105°C in an aluminium tray till constant weight usually 3-4 days, dry weights were determined and clean ring dry weight values, subtracted from dry soil dry values. Bulk density measurements were reported on a $g^{dry\ soil}$ per cm^3 basis, to aid carbon stock calculations (Rowell 2014).

All carbon and nitrogen stock values shown were calculated based on the replicate layer depth measurements, from the plots of each treatment, using a 30 cm normalization value for the maximum depth of the B layer (we observed that the mean depth to parent material was 27.4 cm). These values were then computed with average bulk density measurements for each layer, and again computed using a mean value of each layer, for each treatment, based on the average of eight replicate soil C and N percentage values measured using elemental analyser. No pre-treatment for carbonates was necessary as the soils had a pH ranging from 3.18 - 6.83. pH was measured in 0.01 CaCl with Inolab pH meter with a soil to solution ratio of 1:5, with 2g of air dried soil. There were no significant differences between the pH values between control and +N treatments, measured in both the A and B horizons (Appendix).

Soil respiration amounts and the respective isotopic ratios were measured from samples taken in summer and autumn in 2016 and 2017. Soil respiration was measured only in samples of the A horizon. Soils were sieved through a 3 mm sieve on site and returned to the laboratory on arrival samples were immediately stored at 4°C and measured at 20°C in the laboratory as described above. Soil respiration was also measured in the field using a handheld infra red gas analyser device connected to 13 cm diameter static chambers.

Gross rates of N transformation processes were determined using the ^{15}N isotope pool dilution (IPD) technique (Prommer et al., 2014). ^{15}N additions were calculated in lab pre-experiments. ^{15}N tracer was added which equated to 5µg N g $soil^{-1}$. Both labelled potassium nitrate and ammonium chloride were added separately at different ends of each of the plots, to determine gross mineralization and gross nitrification separately. Label was applied in situ field on each replicate plot using a bespoke 7, 2ml injection device, diameter 8 cm, soil injection volume was approximately 100 ml and it was marked at the center. Soil sampling was conducted at 4 and 24 hours after injection, taking three 0.7 by 4 cm cylindrical cores from randomly across the injection site. Soils from the cores were combined and immediately sieved 3 mm and subsampled a to give a 2 g homogeneous sample, which was mixed with 15 mL of 0.5 M K_2SO_4 shaken for 1 hour then filtered. Sub-samples for soil moisture determination were taken simultaneously. Extracts were immediately stored at 4°c kept for further analyses. Ammonium and nitrate were determined calorimetrically as described above and isotope ratios in the extracts were determined using a modification of the micro-diffusion technique (Sorensen and Jensen 1991) and were measured using EA-IRMS a full series of standards were included in each run.

Modelling.

The experts at Umweltbundesamt were working in close cooperation with the LandscapeDNDC model developers from the Karlsruhe Institute of Ecology (KIT). A technical workshop with participants from both organisations has been carried out from 14. to 15. March 2017. In addition, the Wood-N-Climate consortium was involved in the modelling workpackage of the EU Horizon 2020 project Ecopotential where "Ecosystem Carbon Modelling" was discussed in the relevant panels and events. Lessons learned from all of these activities fed the respective manuscripts. Modelling at the plot scale: For

calibration of LandscapeDNDC, we used several observational data sets from the LTER Zöbelboden site (https://deims.org/8eda49e9-1f4e-4f3e-b58e-e0bb25dc32a6). Data from two long-term monitoring plots were used for the calibration of tree growth (IP1: 1996-2010 and IP2: 1998-2016), and soil respiration (IP1: 2009-2011; IP2: 2015).

Modelling at the landscape scale: The National Park region was discretized by a regular 100 x 100 m grid resulting in a total of 15,793 simulation units. The vertical discretization of the soil and canopy domain was grid cell specific depending on vegetation and soil characteristics available from surveys. The height of the canopy domain was dynamically calculated depending on the maximum height of prevalent vegetation cohorts. The vertical resolution of the soil domain depended on total soil depth but generally 0.5 cm layers were set for upper soil (O and A horizons) and 10 cm layer dimension for lower soil (B horizon). Hourly simulations spanned 15 years covering the time period 2000-2014.

1.1.1 Description of the results and project milestones (also on work package basis)

7 Arbeits- und Zeitplan

Publication of further papers are planned in the next year. Kathrin Schmittner, plans to submit in the Winter Term.

8 Publikationen und Disseminierungsaktivitäten

Tabellarische Angabe von wissenschaftlichen Publikationen, die aus dem Projekt entstanden sind, sowie sonstiger relevanter Disseminierungsaktivitäten.

Stable isotope probing of microbial phospholipid fatty acids in environmental samples. Andrea Watzinger and Rebecca Hood-Nowotny *In*. Stable Isotope Probing: Methods and Protocols: (Eds.) Marc Dumont, PhD and Marcela Hernandez-Garcia, PhD. Springer. New York USA.

SIMSUG 2019, Bristol. Oral presentation: The impact of elevated nitrogen-inputs on organic matter decomposition in Austrian forest soils, determined using a tool-box of stable isotope labelling techniques. Rebecca Hood-Nowotny, Kathrin Schmittner, Andrea Watzinger, Markus Gorfer, Ulf Grandin, Nathalie Korboulewsky, Jutta Stadler, Ika Djulic, and Thomas Dirnböck

EGU2019-16582 Oral presentation: The impact of elevated nitrogen-inputs on organic matter decomposition in European forest soils, determined using a tool-box of stable isotope labelling techniques. Rebecca Hood-Nowotny, Kathrin Schmittner, Andrea Watzinger, Markus Gorfer, Ulf Grandin, Nathalie Korboulewsky, Jutta Stadler, Ika Djulic, and Thomas Dirnböck

EGU2018-5031 Impacts of nitrogen deposition on forest biogeochemical processes using across a trans-European gradient investigated using a tool kit of stable isotope methods (Poster). Kathrin Schmittner, Andrea Watzinger, Markus Gorfer, Ulf Grandin, Nathalie Korboulewsky, Rob Rose, Jutta Stadler, Ika Djulic, Thomas Dirnböck, and Rebecca Hood-Nowotny.

Gesellschaft für Ökologie. 10. bis 14. September 2018 Wien, Österreich. Impacts of nitrogen deposition on forest biogeochemical processes using across a trans-European gradient investigated using a tool kit of stable isotope methods (Oral presentation). Kathrin Schmittner, Andrea Watzinger, Markus Gorfer, Ulf Grandin, Nathalie Korboulewsky, Rob Rose, Jutta Stadler, Ika Djulic, Thomas Dirnböck, and Rebecca Hood-Nowotny.

ISOECOL 2018 Chile: 30 July — 3 August 2018, JH622SL Soil isotope ecology Poster session 1 Mon 30.7.18 19:00 - 21:00 Impacts of nitrogen deposition on forest biogeochemical processes measured in new pools, across a trans-European gradient, investigated using a tool kit of stable isotope methods (Poster) Kathrin Schmittner, Ingrid Rabistch, Andrea Watzinger, Markus Gorfer, Ulf Grandin, Nathalie Korboulewsky, Rob Rose, Jutta Stadler, Ika Djulic, Thomas Dirnböck, and Rebecca Hood-Nowotny.

Stable isotope tools reveal nitrogen deposition effects on forest biogeochemical processes using across a trans-European gradient investigated using a tool kit of stable isotope methods (Oral presentation). Kathrin Schmittner, Andrea Watzinger, Markus Gorfer, Ulf Grandin, Nathalie Korboulewsky, Rob Rose, Jutta Stadler, Ika Djulic, Thomas Dirnböck, and Rebecca Hood-Nowotny. Stable Isotope Network Austria, 16th Stable Isotope Network Austria Meeting. Graz.

Drivers of decomposition in forest soils: Insights from a trans-European experiment, LTER Austria Meeting. Poster (2. und 3. Mai 2017); Ika Djulic, Andrea Watzinger, Markus Gorfer, Ulf Grandin, Nathalie Korboulewsky, Rob Rose, Jutta Stadler, Thomas Dirnböck, and Rebecca Hood-Nowotny

Bodenforum Österreich – ÖBG- Herbsttreffen 2017: Boden im Zentrum von Kreisläufen ÖBG- Tulln. Decomposition in forest soils: Insights from a trans-European experiment (Poster). Andrea Watzinger, Markus Gorfer, Ulf Grandin, Nathalie Korboulewsky, Rob Rose, Jutta Stadler, Ika Djulic, Thomas Dirnböck, and Rebecca Hood-Nowotny

Using stable isotopes to determine of decomposition in forest soils: Insights from a trans-European experiment, LTER Austria Meeting (2. und 3. Mai 2017); Ika Djulic, Andrea Watzinger, Markus Gorfer, Ulf Grandin, Nathalie Korboulewsky, Rob Rose, Jutta Stadler, Thomas Dirnböck, and Rebecca Hood-Nowotny

EGU2017-17859 Drivers of decomposition in forest soils: Insights from a trans-European experiment (Poster). Rebecca Hood-Nowotny et al.,

Talk at CCCA Klimatag in Wien (22. – 24. Mai 2017) - "Impacts of N deposition and climate on forest soil carbon - A model verification study"

ACRP in Essence, Wood-N-Climate. ACRP-Projekte vom Klima- und Energiefonds. Dr. Rebecca Hood-Nowotny.

Schmittner, K; Watzinger, A; Gorfer, M; Grandin, U; Korboulewsky, N; Rose, R; Stadler, J; Djulic, I; Dirnböck, T; Hood-Nowotny, R (2018): Impacts of Nitrogen Deposition on Forest Biogeochemical Processes across a trans-European gradient investigated using a toolkit of stable isotope methods. [Poster] [3rd Student Conference, Department of Forest and Soil Sciences, Vienna, Austria, May 25, 2018] In: Rewald, B., 3rd Student Conference, Department of Forest and Soil Sciences, 2018 - Book of Abstracts; ISBN: 978-3-900932-56-5

Schmittner, K; Watzinger, A; Gorfer, M; Grandin, U; Korboulewsky, N; Rose, R; Stadler, J; Djukic, I; Dirnböck, T; Hood-Nowotny, R; (Poster) (2017): Impacts of nitrogen on forest biogeochemical processes across trans-European gradient investigated using a tool kit of stable isotope methods. 15th Stable Isotope User Group Meeting, University of Vienna, Nov 24-25, 2017. In: Stable Isotope Network Austria, 15th Stable Isotope User Group Meeting

Measuring Carbon and Nitrogen transformation processes across a trans-European gradient, using a toolkit of stable isotope methods (May 2016). Guest Seminar. Institute of Forest Ecology (IFE) Forestry of the BOKU.

Published papers with Wood-N-Climate acknowledgement

Schmitz, A., Sanders, T., Bolte, A., Bussotti, F., **Dirnböck, T**., Johnson, J., Penuelas, J., Pollastrini, M., Prescher, A.-K., Sardans, J., Verstraeten, A., de Vries, W., 2019. Responses of forest ecosystems in Europe to decreasing nitrogen deposition. Environmental Pollution 244, 980-994.

Perring, M.P., Diekmann, M., Midolo, G., Schellenberger Costa, D., Bernhardt-Römermann, M., Otto, J.C.J., Gilliam, F.S., Hedwall, P.-O., Nordin, A., **Dirnböck, T**., Simkin, S.M., Máliš, F., Blondeel, H., Brunet, J., Chudomelová, M., Durak, T., De Frenne, P., Hédl, R., Kopecký, M., Landuyt, D., Li, D., Manning, P., Petřík, P., Reczyńska, K., Schmidt, W., Standovár, T., Świerkosz, K., Vild, O., Waller, D.M., Verheyen, K., 2018. Understanding context dependency in the response of forest understorey plant communities to nitrogen deposition. Environmental Pollution 242, 1787-1799

Submitted publications or in preparation

WP5:

Sonja Leitner, Thomas Dirnböck, Johannes Kobler, Sophie Zechmeister-Boltenstern. Legacy effects of drought on nitrate leaching in a temperate mixed forest on karst. Will be submitted by June 2019.

Abstract: With climate change the occurrence of summer droughts is expected to increase in Central Europe. This could lead to increased nitrate (NO_3^-) export when water scarcity affects the N-uptake capacity of trees and increases soil N mineralization rates due to early leaf senescence and higher litter input. In the present study, we used 16 years of ecological monitoring data from the LTER research site "Zöbelboden" in Austria. The monitoring site is a mixed Spruce-Maple-Ash-Birch forest on karst, which is representative for many watersheds that supply drinking water in Austria. We found that in the year after a summer drought, NO_3^- export via seepage water was significantly elevated compared to the long-term mean. While in normal years, NO_3^- export was primarily controlled by seepage water volume, after a summer drought these controls changed and NO_3^- export was determined by NO_3^- input via precipitation, tree N uptake, and vapor-pressure deficit. Furthermore, higher aboveground litter input during dry years was correlated with increased NO_3^- export in the following year. Our findings show that NO_3^- export from temperate mountain forests on karst is susceptible to summer drought, which could affect drinking water quality in the Central European Alps in the future, especially in combination with forest disturbances like bark beetle outbreaks, which are often a direct consequence of drought damage to trees.

Dirnböck, T., Kraus, D., Grote, R., Klatt, S., Kobler, J., Schindlbacher, A., Seidl, R., Thom, D., Kiese, R. Woody and non-woody understory effects on carbon sequestration in a disturbed European mountain forest landscape. Submitted to Landscape Ecology (2019-03-14).

Abstract: Context: The contribution of forest understory to the temperate forest carbon sink is not well known, increasing the uncertainty in C cycling feedbacks on global climate as estimated by Earth System Models. Objectives: To quantify the effect of woody and non-woody understory vegetation on net ecosystem production (NEP) for a forested area of 158 km² in the European Alps. Methods: We simulated C dynamics for the period 2000-2014, characterized by above-average temperatures, windstorms and a subsequent bark beetle outbreak for the area, using the regional ecosystem model LandscapeDNDC. Results: In the entire study area, woody and non-woody understory vegetation caused between 16 and 37% higher regional NEP as compared to a bare soil scenario over the 15-year period. The mean annual contribution of the understory to NEP was in the same order of magnitude as the average annual European (EU-25) forest C sink. After wind and bark beetle disturbances, the understory effect accelerated, leading to an increase in NEP between 35 and 67% compared to simulations not taking into account these components. Conclusions: Our findings strongly support the importance of processes related to the understory in the context of the climate change mitigation potential of temperate forest ecosystems. The expected increases in stand replacing disturbances due to climate change call for a better representation of understory vegetation dynamics and its effect on the ecosystem C balance in regional assessments and Earth System Models.

Planned publications:

Schmittner, K; Watzinger, A; Gorfer, M; Grandin, U; Korboulewsky, N; Rose, R; Stadler, J; Djukic, I; Dirnböck, T; W, Wanek, Hood-Nowotny R. Impacts of nitrogen on forest carbon and nitrogen turnover across trans-European gradient investigated using a tool kit of stable isotope methods. Paper under final review, Frontiers in Plant Science.

Gorfer M, Djukic I, Dirnböck T, Berger H, Hood-Nowotny R. Soil fungal communities and enzymatic activities from a long-term N-deposition experiment. In prep.

Hood-Nowotny R , Schmittner, K; Watzinger, A; Gorfer, M; Grandin, U; Korboulewsky, N; Rose, R; Stadler, J; Djukic, I; Dirnböck, T; Hood-Nowotny R. Impacts of reactive nitrogen on forest biogeochemical processes in an Austrian forest using a labelled litter bags and isotope pool dilution studies. Paper in preparation for submission to Soil Biology and Biochemistry. Pending, May 2020.

Schmittner, K. Masters thesis. Impacts of nitrogen on forest carbon and nitrogen turnover across trans-European gradient investigated using a tool kit of stable isotope methods. In preparation for submission to University of Vienna, Winter Semester 2019.

Schmittner, K; Watzinger, A; Gorfer, M; Grandin, U; Korboulewsky, N; Rose, R; Stadler, J; Djukic, I; Dirnböck, T; W, Wanek, Hood-Nowotny R. Impacts of nitrogen on forest soil amino acid concentrations. Paper in preparation for submission to Austrian Journal of Forest science.

Diese Projektbeschreibung wurde von der Fördernehmerin/dem Fördernehmer erstellt. Für die Richtigkeit, Vollständigkeit und Aktualität der Inhalte sowie die barrierefreie Gestaltung der Projektbeschreibung, übernimmt der Klima- und Energiefonds keine Haftung.

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