



Universität für Bodenkultur Wien
University of Natural Resources
and Applied Life Sciences, Vienna



**University of Natural Resources
and Life Sciences, Vienna**
Department of Forest and Soil
Sciences
Institute for Soil Research

Ectomycorrhizas and soil nitrogen dynamics in Mongolian forests

Master Thesis

Submitted by

Khatanbaatar Dashbat, BSc

Supervisor:

Univ. Prof. Dr. Douglas L. Godbold Ph.D. Institute of Forest Ecology, University of Natural Resources and Life Sciences Vienna,
Vienna, Austria

Co-Supervisor:

Dr. Burenjargal Otgonsuren
Department of Ecology, Mongolian University of Life Sciences,
Ulaanbaatar, Mongolia

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Statutory Declaration

I herewith declare that I have completed the thesis myself on the subject “Ectomycorrhizas and soil nitrogen dynamics in Mongolian Forests” and not used any sources other than those cited in the literature references. In each individual case, I have indicated the passages that have been taken from other works in terms of their wording or meaning by citing sources or references. I further confirm that this written work has not yet been submitted anywhere.

March 2021

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Abstract

Mongolia's forests are located in a unique transition zone between the Siberian taiga forest and the Central Asian steppe-zone. The climate is extremely harsh with cold, long winters, and the growing season is short and dry. The studies aimed to better understand N dynamics in Mongolian boreal forest soils. This is one of the first investigations of N cycling on Mongolian forests. The N-mineralization and N availability in soils under four tree species were determined at two locations. The soil samples were collected from a depth 0-10 cm of the uppermost soil layer (O horizons) from under each of the species, *Pinus sylvestris* (Scots pine), *Pinus sibirica* (Siberian pine), *Larix sibirica* (Siberian larch) and *Betula platyphylla* (Japanese white birch) at Bogd-Khan Mountain Nukht and Batsumber forests. Soil sampling was carried out in March, June, October and August (2019) in the two forest types. N-mineralization was assessed in a laboratory under controlled conditions and an *in situ* field incubation method. Abiotic and biotic parameters were examined to identify differences between forest trees. These factors can influence the N cycle in the soil. For example, temperature, C/N-ratio, and water content were correlated with net N-mineralization of NH_4^+ and NO_3^- as essential variables influencing biogeochemical processes. The mineralization rates of NH_4^+ and NO_3^- were higher in *Larix sibirica* and *Betula platyphylla* as compared to *Pinus sylvestris* and *Pinus sibirica*. The results showed that N availability is high when the soil thaws in spring or when rainfall increases in autumn. The nitrogen availability varies depending on the tree species (*Pinus sylvestris* > *Pinus sibirica* > *Betula platyphylla* > *Larix sibirica*). N availability showed a general decrease over the two years of the investigation. This study identified 23 ectomycorrhizal morphotypes associated with *Pinus sylvestris*, *Pinus sibirica*, *Larix sibirica* and *Betula platyphylla*. Several of the morphotypes were common to all tree species.

Keywords: Soil N-mineralization, soil N availability, Ectomycorrhiza, *in situ* incubation

Kurzfassung

Die Wälder der Mongolei befinden sich in einer einzigartigen Übergangszone zwischen dem sibirischen Taiga Wald und der zentralasiatischen Steppenzone. Das Klima ist extrem rau mit kalten, langen Wintern, und die Vegetationsperiode ist kurz und trocken. Das Ziel der Masterarbeit ist es, die N-Dynamik in mongolischen, borealen Waldböden besser zu verstehen. Dies ist eine der ersten Untersuchungen zum Stickstoffkreislauf in mongolischen Wäldern. Die N-Mineralisierung und N-Verfügbarkeit in Böden unter vier Baumarten wurde an zwei Standorten bestimmt. Die Bodenproben wurden aus einer Tiefe von 0-10 cm der obersten Bodenschicht (O-Horizonte) unter *Pinus sylvestris* (Waldkiefer), *Pinus sibirica* (sibirische Kiefer), *Larix sibirica* (sibirische Lärche) und *Betula platyphyla* (Birke) in den Wäldern von Bogd-Khan Mountain Nukht und Batsumber entnommen. Die Bodenproben wurden jeweils im März, Juni und Oktober 2018 sowie im August 2019 entnommen. Die N-Mineralisierung wurde in einem Labor unter kontrollierten Bedingungen und einer *in-situ* Feld-Inkubationsmethode bewertet. Abiotische und biotische Parameter wurden untersucht, um Unterschiede zwischen Waldbäumen zu identifizieren. Faktoren, welche den Stickstoffkreislauf im Boden beeinflussen können sind: Temperatur, pH-Wert und Wassergehalt. Deren Bedeutung wurde mit der Netto N-Mineralisierung von NH_4^+ und NO_3^- als wesentliche Einflussgrößen biogeochemischer Prozesse bestätigt. Die Mineralisierungsraten von NH_4^+ und NO_3^- Raten waren in Böden unterhalb von sibirische Lärche und Birke im Vergleich zu Waldkiefer und sibirische Kiefer höher. Die Ergebnisse zeigten, dass die Stickstoffverfügbarkeit hoch ist, wenn der Boden im Frühjahr auftaut oder wenn die Niederschläge im Herbst zunehmen. Die Stickstoffverfügbarkeit variiert in Abhängigkeit von der Baumart (Waldkiefer > Sibirische Kiefer > Birke > Lärche). In dieser Studie wurden 23 Ektomykorrhiza-Morphotypen identifiziert, die mit *Pinus sylvestris*, *Pinus sibirica*, *Larix sibirica* und *Betula platyphyla* assoziiert sind. Mehrere der Morphotypen waren für alle Baumarten gemeinsam.

Schlüsselwörter: N-Mineralisierung im Boden, N-Verfügbarkeit im Boden, Ektomykorrhiza, *in situ* Inkubation

Хураангуй

Монгол орны ой нь Сибирийн тайгын үргэлжлэл бөгөөд Төв Азийн тал хээр, цөлийн бүсийн хоорондох шилжилтийн өвөрмөц бүсэд оршдог. Монгол орон байгаль цаг уурын эрс тэс уур амьсгалтай, өвлийн улирал нь урт, ургамал ургалтын хугацаа богино, хур тунадас бага унадаг, улирлын температурын ялгаа их байдаг онцлогтой. Уур амьсгалын өөрчлөлт болон хүний сөрөг үйл ажиллагааг бууруулах зорилгоор төрөөс ойн бодлого төлөвлөгөөнүүдийг боловсруулан ажиллаж байгаа хэдий ч ойн хөрсний үүрэг, ач холбогдлыг орхигдуулж байна. Энэхүү судалгааны ажлаар Монгол орны ойн хөрсний азотын динамикийг тодорхойлох зорилго дэвшүүлсэн. Монгол орны ойн азотын эргэлтийн судалгааг анх удаа хийж байгаа нь уг ажлын шинэлэг тал юм. Ойн хөрсний азотын эрдэсжилт, азотын агууламжийг Богд Хаан уулын Нүхтийн амны нарс-хушин холимог ой болон Батсүмбэр сумын Үдлэгийн амны шинэс-хусан холимог ойд тодорхойлсон. Нарс (*Pinus sylvestris*), Хуш (*Pinus sibirica*), Шинэс (*Larix sibirica*), Хус (*Betula platyphylla*) гэсэн 4 зүйлийн моддын хөрснөөс 0-10 см хүртлэх гүнээс дээж авч азотын эрдэсжилтийг лабораторид хяналттай нөхцөлд болон талбайд *in situ* инкубаци хийх аргуудыг ашиглан судалгааг явуулсан. Дээж талбайн ялгааг тодорхойлохын тулд абиотик, биотик үзүүлэлтүүдийг авч үзэв. Судалгааны үр дүнгээс харахад хавар хөрс гэсэх мөн намар хур тунадасны хэмжээ нэмэгдэхэд нитратын агууламж өндөр байсан. Хөрсний усны агууламж нь экосистем дэх азотын эргэлт болон азотын хүртээмжтэй байдалд нөлөөлдөг болохыг судалгааны үр дүн харуулж байна. Модны төрөл зүйл, хөрсний чийг, pH зэрэг үзүүлэлтээс хамаарч азотын агууламж харилцан адилгүй байна (Нарс > Хуш > Хус > Шинэс). Уг судалгаагаар Нарс, Хуш, Шинэс, Хус моддын үндэснээс эктомиокоризын 23 өөр морфотипийг тодорхойлов. Бид энэ судалгаагаараа Монгол орны ойн экосистемийн үйл ажиллагаанд хөрс нь гол үүрэгтэй болохыг үзүүлсэн болно. Цаашид Монгол орны ойн хөрсний шинж чанар, ургамалжилт, бичил биетний үйл ажиллагаа, ойн модны харилцан үйлчлэлийг цогц байдлаар хамтад нь нарийвчлан судлах шаардлагатай байна.

Түлхүүр үг: Азотын эрдэсжилт, хүртээмжтэй байдал, эктомиокориза, *in situ* талбайн инкубаци

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Abbreviations

ANOVA	One way analysis of similarities
a.s.l.	above sea level
BH	breast height
C	Carbon
CEC	cation exchange capacity
Corg	organic carbon
DOC	dissolved organic carbon
DOM	dissolved organic matter
DON	dissolved organic nitrogen
EM	Ectomycorrhizas
Fig	Figure
MB-C	Microbial biomass carbon
MB-N	Microbial biomass nitrogen
N	Nitrogen
NH ₄ ⁺	Ammonium
NO ₃ ⁻	Nitrate
P	Phosphorus
SE	Standard Error
T0	Measurement before incubation
T1	Measurement after incubation
TN	Total nitrogen
UNESCO	United Nations Educational, Scientific and Cultural Organisation
°C	Celsius degree
μ	micro

The Cyrillic script is not presented in the text of this study. The transcription into Cyrillic in the alphabet is as shown below.

Mongolian name	Transcription
Батсүмбэр	Batsumber
Богд Хан уул	Bogd-Khan Mountain
Нүхт	Nukht
Төв Аймаг	Aimag (English: province)
Сум	Sum
Үдлэг	Udleg
Улаанбаатар	Ulaanbaatar
Хангай	Khangai Mountains
Хэнтий	Khentey Mountains

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1 Introduction

Forests in Mongolia

The boreal forest is the second largest biome globally, having 33% of the earth's forests cover (FAO., 2001), and plays an essential role in the protection of the earth's climate. The boreal forest, also known as taiga, stretches right across the northern hemisphere over three continents and covers ten countries (Osman., 2013). This forest type is characterized by short summers and long cold winters (Högberg et al., 2017). In Mongolia, the northern part of the country is covered by boreal forest with a transition to a forest-steppe in the south. A unique feature of the forest-steppe on the southern edge of the Mongolian boreal forest is that the forests are naturally fragmented, and they occupy the wettest locations, which are essentially north-facing mountain slopes, while dry locations are covered with grasslands. The proportion of forested areas covered by forests is 7.9% of the total land area (Ministry of Nature Environment and Tourism., 2019).

The forest area of Mongolia is divided into several zones: 1) the Siberian taiga, 2) the taiga forest of the mountain regions, 3) the coniferous forest of the forest-steppe forming the ecological transition zone between the closed boreal forest and the steppes of Central Asia (Dulamsuren et al., 2009), and 4) the saxaul forest of the Gobi desert region. The boreal forest and the forest of the forest-steppe are dominated by six main species: *Larix sibirica* (Siberian larch), *Betula platyphylla* (Japanese white birch), *Pinus sibirica* (Siberian pine), *Pinus sylvestris* (Scots pine), *Populus tremula* (Quaking aspen) and *Picea obovata* (Siberian spruce), with much of the forest-steppe being dominated by larch forests (Tsogtbaatar., 2004) but can include several tree species (REDD+Mongolia., 2017). The growth rate of forests is slow because of the harsh continental climate, which is much harsher than in other countries within a similar latitudinal zone and characterized by extremes in both absolute temperature and temperature fluctuations. Precipitation is concentrated in the summer period, and has a low with an annual average of about 230 mm, but is higher in mountainous areas in the north zone, about 330 mm (Dulamsuren et al., 2010). As a result of the harsh climate, the forest floor is characterized by a slow decomposition rate (Read et al., 2004), and the natural regeneration capacity is extremely limited. These forests are strongly affected by fires, pests and human activities. In recent years, deforestation has decreased due to forest and steppe fires, extreme drought stress (Dulamsuren et al., 2009), non-technological forest use, and biological patterns of insect reproduction. The study areas in this work are located in the drier and coldest part of

Mongolia. In this area, the boreal taiga-Siberian climate ends, and the steppe climate begins, which then further extends into the Gobi region (Fig. 2, Fig. 3).

Ecosystem processes

Two important parameters for the function and productivity of ecosystems are the dynamics of the microbial community and the nitrogen (N) cycle (Fig. 1), (McMillan et al., 2007).

Ectomycorrhiza

In Mongolia, soil fungus research has been carried out to a certain extent since the 1970s. Although mycorrhizal studies have been conducted since 1999, there have been few studies on ectomycorrhiza (EM). Otgonsuren et al. (2020) has shown that viable winter EM give *Pinus sibirica* and *Pinus sylvestris* an advantage in obtaining nutrients when the soil thaws in spring. Another study by Otgonsuren. (2013) demonstrates that EM fungi can effectively form EM with *Pinus sylvestris* (scots pine) seedlings and improve their growth ability and freezing tolerance.

Mycorrhiza diversity and plant growth, as well as research into the beneficial effects of exposure, have recently begun. Soil organisms take over essential functions in the forest soil. They are responsible for litter decomposition, humus formation, and the supply of nutrients. An important function of soil organisms is the partnership in mutualistic symbiosis with plants (Smith and Read., 2008). In forested ecosystems where N is a major limiting nutrient, as in boreal forests, it is thought that EM fungi play a crucial role for plant N acquisition and tree growth (Read et al., 2004). All of the trees found in the Boreal and steppe forest of Mongolia are EM forming species (Smith and Read., 1997). EM fungi may be particularly important in dry, N limited forest ecosystems, as they can efficiently absorb nutrients (N and P) and as well as water for the host plant (Qu et al., 2010). It has been suggested that up to 75% of phosphorus (P) and 80% of nitrogen (N) acquisition is facilitated by mycorrhizal fungi (Smith and Read., 2008). EM fungi constitute up to 40% of the total soil microbial biomass C and contribute substantially to autotrophic CO₂ respiration (Rosinger et al., 2018). Several studies have shown that up to 68% of the photosynthetically derived carbon (C) is allocated to EM fungi for growth and respiration (Hobbie et al., 2006).

Nitrogen cycle

Carbon, phosphorus, sulfur, and nitrogen are the most critical elements for living organisms and for the functioning of ecosystems. Nitrogen plays an essential role in the stability of ecosystems and their diversity (Jussy et al., 2004), and in the nutrition and productivity of forest stands (Nadelhoffer et al., 1985). Many forest ecosystems are characterized by the growth-limiting availability of the macro-nutrient N (Rennenberg et al., 2009). In N limited forests, recycling of N through biomass cycles is particularly important for N nutrition (Rennenberg et al., 2009). N stored by plant and microbial biomass is converted back into available forms, primarily ammonium and nitrate, through mineralization (Fig. 1). Temperature and the quality of plant litter are important limiting factors of availability of N for plants in the boreal forests. The soil is dominated by cold temperatures and litter inputs are mostly recalcitrant in nature, thus rates of organic matter decomposition are low. But N- mineralization and nitrification rates also depend on several factors, such as moisture conditions (Gadgil and Gadgil., 1978) and the availability of labile organic matter for the conversion into the inorganic forms of ammonium (NH_4^+) or nitrate (NO_3^-) (Jansson and Persson., 1982). Therefore, it is essential to understand the cycling of soil N in forest ecosystems to estimate the effects of global climate change on C cycles, and for making management and conservation decisions in boreal forests (Alexandra et al., 2013).

Measurements of the net N-mineralization rate in forest soils generally aim to provide an index that can be correlated with the nutrient cycle. Several methods can be used to determine soil net N-mineralization, including laboratory-based methods using incubation under controlled temperatures (Knoepp and Swank., 1998), and *in situ* incubation methods in the (Deluca et al., 2002). In both cases, the soils are incubated for several weeks. *In situ* methods use sealed bags of soil reinserted into the soil profile, but also more durable covered-cylinders. The cylinders are usually constructed of PVC or metal pipes that are capped to exclude rainfall (Hanselman et al., 2004). For Nukht sites, 20 core pairs are established to adequately characterize N-mineralization in an area.

The forest ecosystem of the soil N cycle consists of three processes: Input, Transformation and Output. These processes include biological nitrogen fixation, litter decomposition, N-mineralization, nitrification, denitrification, N oxide emission and leaching (Fig. 1). Net N-mineralization is measured in that by putting the soil in bags or cylinders or using laboratory incubations of soil, the uptake of N via plants is eliminated. Hence the method measures the amounts of ammonium (NH_4^+) or nitrate (NO_3^-) produced minus the amount that has been immobilized in the microbial biomass.

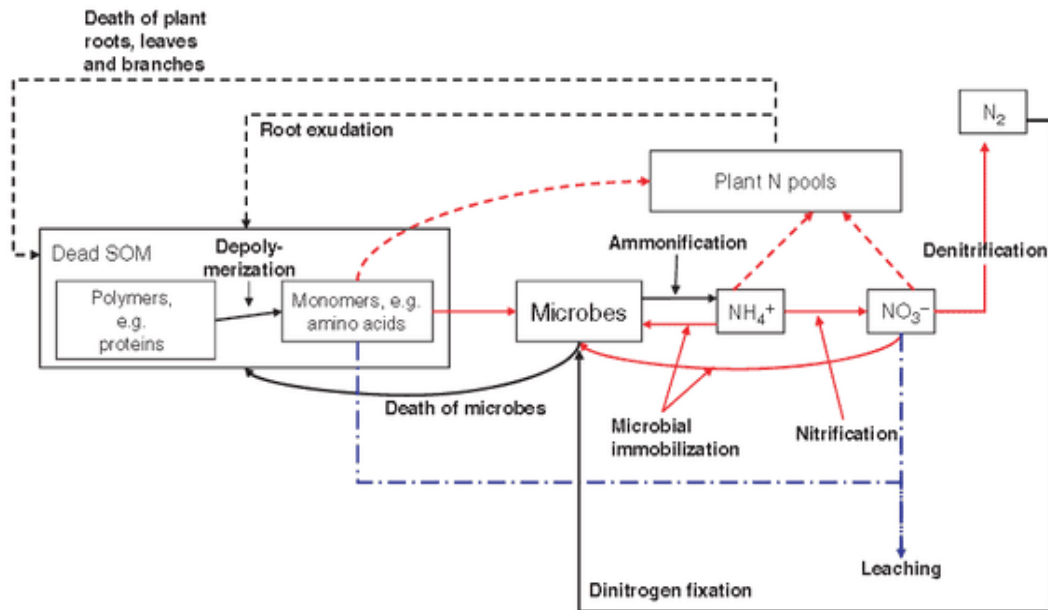


Figure 1: Simplified diagram of the nitrogen cycle. SOM: soil organic matter. Dashed arrows indicate plant processes and, solid arrows: microbial processes, solid and red dashed arrows: competitive processes between plants and microorganisms, blue arrows: hydrological transport pathways (Rennenberg et al., 2009).

2 Objectives

This master's thesis is an investigation of the role of N availability in ecosystem processes in the south-westerly boreal forest of Mongolia. In particular, the N was chosen as the one nutrient most likely to limit plant growth. The aims are to evaluate soil chemical characteristics, net N-mineralization, ammonification, and nitrification between the different forest types one composed of *Pinus sibirica*, and *Pinus sylvestris*, and one composed of *Larix sibirica* and *Betula platyphylla*. The investigations included both *in situ* (2019 Nukht) and laboratory estimations.

Specific objectives

- To determine soil physical and chemical properties of soil characteristics of organic layers and mineral soils.
- To estimate the total nitrogen dynamics for the years 2018 and 2019.
- To assess the ectomycorrhizas of the different tree species.

3 Site descriptions

3.1 Study sites

Investigations were carried out in two different forest regions near the capital Ulaanbaatar in Mongolia. The research area's mainframe includes four species of trees selected from the Nukht and Batsumber (Udleg) region located in Tuv province. Soil samples were taken at both sites to estimate nitrogen dynamics in boreal forests. The study was conducted in 2018 and 2019.

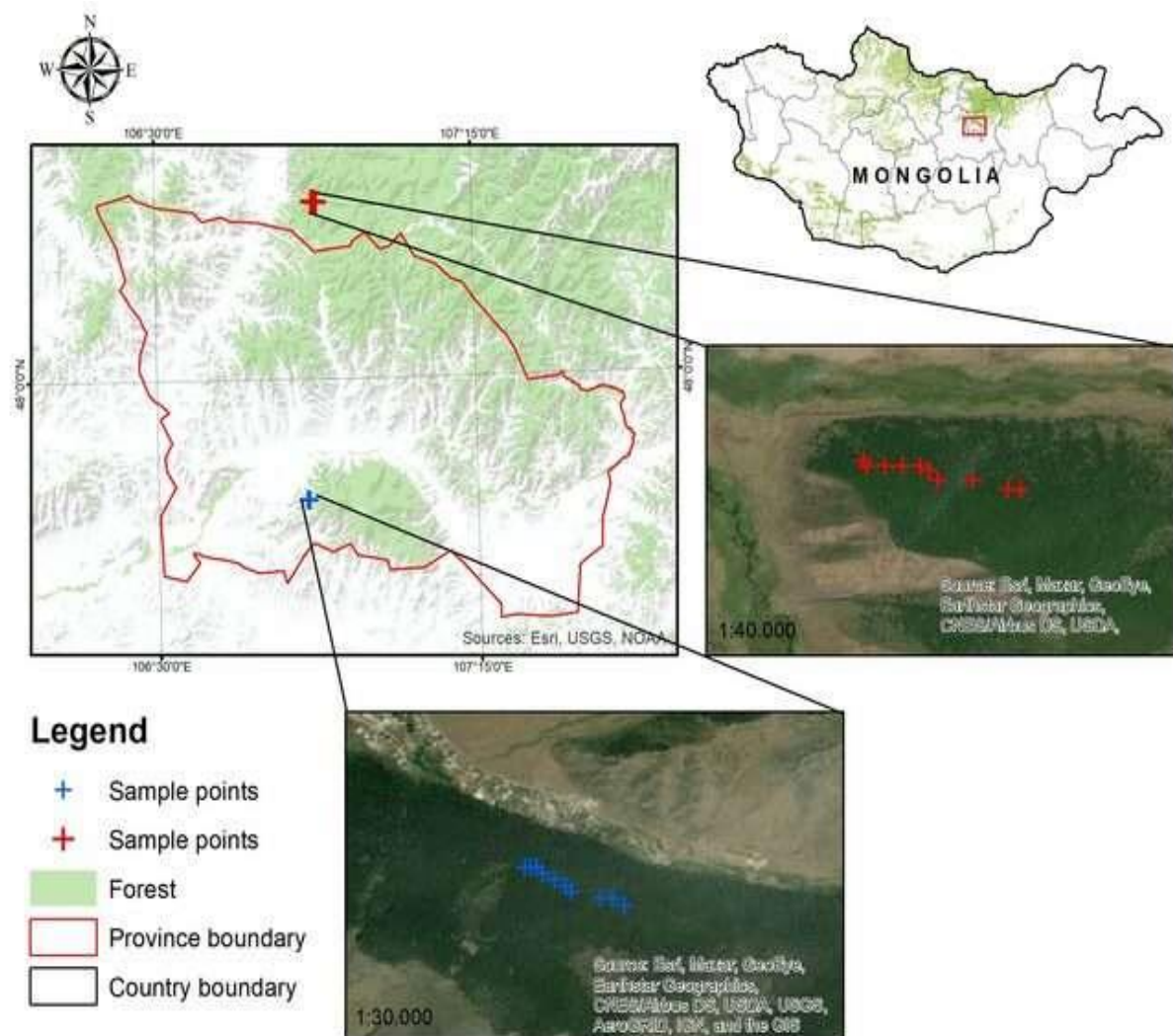


Figure 2: Locations of the study areas. The locations of the sampling points are indicated by a plus sign (Nukht area is in blue, and Batsumber is in red).

3.1.1 Study sites in the Nukht Region

The Nukht valley was selected for the study. It is located southwest of Ulaanbaatar approximately 10 km from the downtown (Fig. 1). The exact location coordinates are 47°49'30" N latitude; E longitude 106°51'00", along the mountain ranges of the Bogd-Khan biosphere reserve. The natural zone of the Bogd-Khan Mountain form the boundary of the steppe and forest-steppe, which is very susceptible to changes in environmental conditions. The Bogd-Khan Mountain is one of the oldest officially protected areas and now part of the UNESCO world heritage list (UNESCO World Heritage., 1996). The soil samples were collected two elevations, 1514 m and 1600 m. The forest is composed of species the *Pinus sibirica* (Siberian pine), *Pinus sylvestris* (Scots pine) and, in some cases, mixed with *Picea obovate* (Siberian spruce). *Pinus sibirica* (Siberian pine) is the most dominant species and is viewed as the potential natural vegetation of the area (TFL., 2018). The shrub layer consists of *Rosa acicularis* (Rosaceae) and regeneration of *Pinus sylvestris*. Tree species in the study area were estimated to be between 100 and 130 years old and had a mean diameter at breast height (DBH) of approximately 28 to 38 cm and height from 18 to 24 m (TFL., 2018).

3.1.2 Climate of Nukht

The areas climate conditions are characteristic to that of the steppe climate (semi-arid climate) and a humid continental climate (Erdenechimeg., 2013). The mean annual air temperature of Bogd-Khan mountain is -0.5 °C. The coldest month when the lowest temperature was recorded was -33.3 °C, on 25 January in 2018, and the highest temperature was 24.9 °C on 31 May. The precipitation amount varies greatly depending on the altitude. At the top of the mountain, precipitation is around 350 mm per year, decreases to about 250 mm at a lower elevation. The months of July and August are likely to receive 80% of the total annual rainfall. In March, the monthly average temperature was -3 °C, in June 17.4 °C and in October 2.9 °C. But in 2019, 18.9 °C in June and 15.1 °C in August. Air temperature data were from www.en.tutiempo.net for weather station at the located International airport ca. 7 km from the Nukht site (Fig. 3a, b).

3.1.3 Study sites at Batsumber (Udleg)

The second research area is located at the Batsumber (Udleg) in Tuv province (48°15'27" N, 106°52'32" E), about 84 km from the Mongolian capital Ulaanbaatar (Fig. 3). In terms of the subdivision of physical geography, the Batsumber region belongs to the Khentey

Mountain Taiga (Tsegmed., 1967). The forest-steppe zone with an elevation of 1270-1400 m is dominated by forests in particularly of *Larix sibirica* (Siberian larch) and *Betula platyphylla* (Japanese white birch) and *Populus laurifolia* (Poplar) mixed stands. The ground vegetation at the research site consists of *Rosa acicularis* (Rosaceae), *Leontopodium* (Edelweiss) and a naturally regenerated *Larix sibirica* (Siberian larch). Tree species were estimated to be between 120 and 60 years old and had a mean diameter at breast height (DBH) of approximately 16 to 32 cm and height from 12 to 14 m (TFI. 2018).

3.1.4 Climate of Batsumber

In the forest-steppe ecotone of the Khentey Mountains in northern Mongolia, soil moisture is strongly influenced by exposition, slope, the presence or absence of permafrost and vegetation cover. Average annual precipitation is 250-400 mm and the spring and autumn periods are dry with a short growing season. The frost-free period lasts about 110-120 days. 81-93% of the amount of precipitation falls in the warm season (from June to October) and 7-19% during the cold season (from November to February). The monthly mean temperature was -2.9 °C in March, in June 18.5 °C and in October was 3.3 °C. Air temperature data were obtained from the Forest Research Training-Center in Udleg (Fig. 3a, b).

4 Methodology

In 2018 and 2019, 20 replicate plots at two different sites were established for each tree species. The area of each plot was approximately 16 m². To obtain areas dominated by *Pinus sibirica*, *Pinus sylvestris*, *Larix sibirica* and *Betula platyphylla* the plots were established in the interspace between the trees in the groups described above. Thus, we analyzed a total of 20 sampling plots, composed of four tree species x 5 plots.

4.1 Fieldwork

The fieldwork was carried out from the end of March, June and October from 2018, (at Nukht) to mid-August 2019. In 2018, a total of 50 soil cores were taken from each of the 20 control plots (both stands) at the end of March, mid-June and early October. The following year in 2019, 50 soil samples were collected in mid-July and at the end of August. The *in situ* mineralization was carried out using a field incubation for 30 days from 23.07.2019 to 20.08.2019.

Table 1: Sampling dates at each location

Date	Location	Tree species	Longitude	Latitude	Elevation
2018 (Mar, Jun, Oct) and 2019 (Jun and Aug)	Nukht	<i>Pinus sibirica</i> and <i>Pinus sylvestris</i>	47 °49'30"	106 °51'00"	between 1514 m to 1600 m
2018 (Jun and Oct) and 2019 (Aug)	Batsumber	<i>Larix sibirica</i> and <i>Betula platyphylla</i>	48 °15'27"	106 °52'32"	between 1278 m to 1316 m
from 23 rd July to 20 th August 2019	Nukht (<i>in situ</i>)	<i>Pinus sibirica</i> and <i>Pinus sylvestris</i>	47 °49'30"	106 °51'00"	between 1514 m to 1600 m

A total of 110 soil samples were taken from Mongolia to Vienna in order to use and examine in the laboratory.

Sample collection, transport, and storage

Soil samples were collected from a depth 0-10 cm of the uppermost soil layer (O horizons) from under each species *Pinus sibirica*, *Pinus sylvestris*, *Larix sibirica* and *Betula platyphylla*, using a 10 cm diameter stainless steel corer and a spade. Solid samples were safely stored in plastic bags, and liquid solutions were prepared in the laboratory of the

Mongolian University of Life Sciences (MULS). The soil samples were transferred frozen to the laboratory at -14 °C until further use. During the sample transportation of the samples from Mongolia to Vienna, the heat and humidity conditions may have changed. For analysis, frozen subsamples were removed from each of the frozen soil blocks and kept at 4 °C overnight.

The soils were collected 4 times in two years (2018 and 2019), soil samples were taken from both sites. Soil O-horizon consists of organic residues, including barks, leaves, twigs and fruits etc. Organic layers were collected and studied using the structure Oi, Oe and Oa layers (Osman et al., 2013).

4.2 Laboratory analyses

The sample analysis was performed at the Institute of Forest Ecology, University of Natural and Life Sciences, Vienna (BOKU). The timeframe of the laboratory investigation was from May 2018 to October 2019. All the samples taken from Mongolia were investigated within 3-4 weeks. Before analysis, the samples were unpacked and stored at standard room temperature. The laboratory investigation generally focused on a set of physical and chemical characteristics essential for the characterization of soil and forest cover conditions.

Table 2: Laboratory methodology and used technical equipment

Method and parameter	Technical equipment	References
Net N-mineralization and nitrification, and <i>in situ</i> incubation	laboratory and tube method	(Raison et al., 1987)
N-mineralization extraction (NO ₃ ⁻ and NH ₄ ⁺)	Microplate 660 nm and nitrate 540 nm	(Schinner et al., 1996) (Miranda et al., 2001)
Soil temperature	(model DS1922L-F5, precision: 0.5 °C, accuracy: ±1 °C)	(<i>In situ</i> soil moisture measurements), (®The iButton)
Gravimetric H ₂ O content at 105 °C	BINDER	(Schinner et al., 1996)
pH-value in H ₂ O and CaCl ₂ and	pH-Meter CG 840, VWR shaker	(Blume et al., 2010)
DOC, DON measurements	Shimadzu TOC 5050 analyser	(Schinner et al., 1996)

C/N-ratio	TruSpec CN analyser	(Leco and Joseph.,)
To determining P	LECO	(Bray and Kurtz. 1945)
An extraction soil microbial biomass	Megafuge 10 centrifug	(Witt et al., 2000)
Microbial biomass	Shimadzu TOC/TN analyser	(Vance et al., 1987)
EM morphotyping	ZEISS (Stemi 2000-SC)	(Agerer., 1997)
	AxioCan ERc5s camera	

4.2.1 Preparation of Extracts and Incubation

The incubation was carried out with 10 g of soils and equally divided into two different tubes to measure net N-mineralization within two different time frames (T0 and T1).

Measurement before incubation (T0) sample is a direct measure. Five grams of sieved soil of each sample was extracted in 50 ml of 1M KCl by shaking for 2 hours on a Rotating Shaker at 20 rotational frequency per minute. Subsamples of the extracts were centrifuged for 5 minutes on the Megafuge 1.0 centrifuge at 4000 rpm and filtered through a Whatman 42 (pore size 2.5 µm) filter paper. Nitrate was determined using the microplate assay of (Miranda et al., 2001) using vanadium (III) as a reductant and determination of nitrite using Griess reagents. Absorbance was determined at 540 nm. Ammonium was determined using the indophenol blue method modified for a 96-well microplate (Schinner et al., 1996). Extinction was determined at 660 nm. DOC and DON were measured on a Shimadzu TOC 5050 analyzer.

Another 5 g (measurement after incubation T1) fresh soil was filled into 50 ml polypropylene tubes and sealed with Parafilm, which allowed air exchange but retarded moisture loss. The tubes were incubated in a growth chamber at 20 °C with 24h light/dark cycle for 30 days (Hart et al., 1994). NH_4^+ and NO_3^- were extracted from the soil and analyzed as described above. The Net N-mineralization was calculated from the inorganic N changes after 30 days of incubation in the growth chamber.

Calculations:

Ammonium:

$$VP * V / EW * \%TS = \mu\text{g N- } \text{NH}_4^+ / \text{g Drymass}$$

VP: sample result (µg/mL)

V: total volume used for extraction (ml)

EW: sample weighed in (g)

$$100 * \%^{-1} TS : \text{dryfactor}$$

Nitrate:

$$(VP * V) / (EW * \%TS) = \mu\text{g N- NO}_3^- / \text{g Drymass}$$

VP: sample result ($\mu\text{g/mL}$)

V: total volume used for extraction (ml)

EW: sample weighed in (g)

$$100 * \%^{-1} TS : \text{dryfactor}$$

Incubation:

$$\text{Net nitrification} = T1 \text{ NO}_3^- - T0 \text{ NO}_3^- - \text{inputs of NO}_3 + \text{leaching of NO}_3^-$$

$$\text{Net ammonification} = \text{gross mineralization} - \text{microbial NH}_4^+ \text{ immobilisation}$$

$$\text{gross nitrification} = T1 \text{ N NH}_4^+ - T0 \text{ NH}_4^+ - \text{inputs of NH}_4^+ + \text{leaching of NH}_4^+$$

$$\text{Net mineralization} = \text{net nitrification} + \text{net ammonification}$$

4.2.2 Soil temperature

Temperature sensors (model DS1922L-F5, precision: 0.5 °C, accuracy: ± 1 °C) were set to record temperatures every 60 min and buried to 10 cm beneath the soil surface. The data were read using a one-wire viewer from on 23rd July to on 20th August 2019. The average daily soil temperature was calculated from hourly measurements.

4.2.3 Determination of water content

The soil moisture content was determined gravimetrically; the weight of the aluminium dish is considered. Five grams of fresh soil was filled through a 2 mm sieve in an aluminium dish and transferred to dry at 105 °C in an oven for 24 hours.

4.2.4 Soil pH measurements, total carbon and total nitrogen

Soil pH was determined at 1:2 (w/w soil: extract) soil suspension in distilled H₂O and a 0.01 M CaCl₂. The pH values were measured using a pH-Meter CG-840.

Approximately 10 g of each sample was diluted in 25 ml of each solution. The samples were then shaken for about 5 minutes. The solutions were left in the refrigerator for 24 hours at 4 degrees. The next day the pH-value was examined at a Schott-pH-meter CG840.

For the determination of total C and total N, soils dried at 105 °C were finely ground in a mortar, and total C and N were measured using automated dry combustion in a TruSpec C/N analyzer (Leco and Joseph).

4.2.5 DOC and DON measurements

To an analysis of dissolved organic carbon (DOC) and nitrogen (DON), 5 grams sieved soil of each sample were extracted with 50 ml 1M KCl by shaking for 2 hours on a Rotating Shaker at 20 rotational frequency per minute. Subsamples of the extracts were centrifuged for 5 minutes on the Megafuge 1.0 centrifuge at 4000 rpm and filtered through a Whatman 42 (pore size 2.5 µm) filter paper. Subsequently, DOC and DON were determined using a Shimadzu TOC/TN analyzer (Schinner et al., 1996).

4.2.6 Determination of Phosphorus

The soil test phosphorus method was developed by Bray and Kurtz (1945). For the analysis of phosphorus, the samples were sieved through a 2 mm sieve before drying, and 2 grams of sieved soil were extracted with 50 ml NH₄F by shaking for 5 minutes on a Rotating shaker, then filtered through Whatman No. 42 filter paper for 1 hour.

$$\text{Available P (mg P/ kg soil or ppm)} = \frac{C_p * 0.02 \text{ (L)}}{0.002 \text{ (Kg)}}$$

C_p= Concentration of P (mg/L) in Bray and Kurtz

0.02 L= 20 ml extract

0.002 Kg = 2 g soil (if amount of soil is different use that value, like 2.1g =0.0021 Kg)

4.2.7 Microbial biomass C and N

The microbial biomass was determined for the soil using fumigation method (Vance et al., 1987). Two sets of 5 grams of moist soil (sub-sample were used for determining moisture content) were placed into 50 ml centrifuge tubes (for non-fumigation) and 20 ml glass scintillation beaker (for fumigation). The non-fumigated reference samples were extracted with 25 ml of 0.5 M K₂SO₄ in 50 ml centrifuge tubes (Witt et al., 2000). After shaking at 200 rpm speed for 1 hour, the extract was centrifuged for 5 min at 4000 rpm speed, then filtered through a Whatman no. 42 filter paper and preserved in 20 ml plastic scintillation vials at 4 °C.

The sample for fumigation (in glass beaker) was placed in a vacuum desiccator. A 100 ml glass beaker with approximately 40 ml of ethanol-free chloroform and anti-bumping granules was placed in the middle of the glass beaker containing soil samples in the desiccator. The desiccator was evacuated with a vacuum pump until the chloroform boiled. The samples were then left in the desiccator in a dark cupboard for 24 hours. After fumigation, the soil was extracted with K_2SO_4 (Vance et al., 1987). The C and N in both fumigated and non-fumigated samples were extracted using a Shimadzu TOC/TN analyzer.

Calculation:

$$\text{Soil microbial biomass C (mg C kg}^{-1} \text{ soil)} = F/K$$

$$F = (\text{Organic C extracted from fumigated soil}) - (\text{organic C extracted from non fumigated soils})$$

$$K = \text{Extraction efficiency} = 0.45 \text{ (generally)}$$

$$\text{Microbial biomass N } 0.54 (K_{EN}) \text{ N respectively}$$

4.2.8 Ectomycorrhizal morphotyping

For assessing the EM community structure, fine roots were carefully removed from each soil subsample taken from the soil. The roots were rinsed with tap water, placed in Petri dishes filled with clean tap water, and stored at 4 °C. All EM root tips that were turgescient and vital in appearance were divided into morphotypes using the method described by (Agerer., 1997), using ZEISS (Stemi 2000CS) dissecting microscope connected to an AxioCam ERc5s camera.

4.3 Statistics

All data results were collected and calculated in Microsoft Excel Files. Statistical analysis of data carried out using the program SPSS 26 (one-way ANOVA). The Tukey test was used as a post-hoc test. Before the analysis, the data were checked for normality. If the data were not normally distributed, they were \log_{10} transformed and reanalyzed. Calculated means and standard errors of the soil parameters from each plot. We describe a P value of ≤ 0.05 as statistically significant and indicate. The results were statistically significant when $P < 0.05$. The tests compared the different forest areas at each of the two locations.

The graphical output was used by SigmaPlot 14.0.

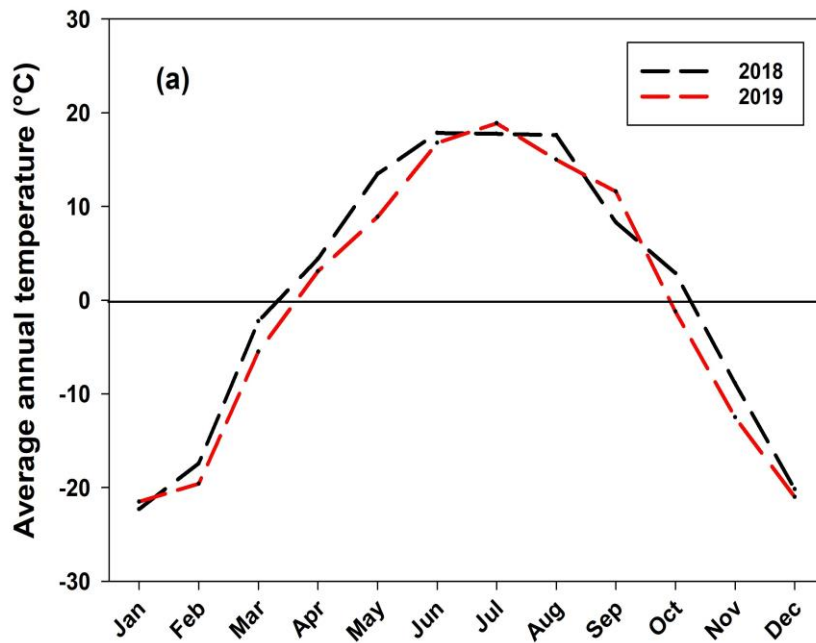
5 Results

5.1 Abiotic soil parameter

5.1.1 Soil and air temperature

The air temperatures at the two sites in 2019 are shown in Figure 3a. The lowest mean air temperature reached was -26.8°C in January at Batsumber, and -22.3°C at Nukht. From March until November the air temperatures were similar at both sites. The highest mean air temperature was 18.6°C in June. Precipitation occurs mainly in the summer months and especially between July to September. The maximum precipitation in July 2018 was 128 mm (Fig. 3b). Figure 3b shown that precipitation in 2019 is relatively lower than in 2018.

The daily soil and air temperatures at Nukht from mid-July to mid-August in autumn 2019 are shown in Figure 3c. Shown are the temperatures during the *in-situ* incubation period. The soil temperatures followed the air temperatures but with a lag of ca. 2 days. The lowest air temperature reached during this period was 11°C in August, and corresponded to the lowest soil temperature was 9°C . The soil temperature varied between 8.9°C and 13.8°C . The average soil temperature during the incubation period was 11.2°C .



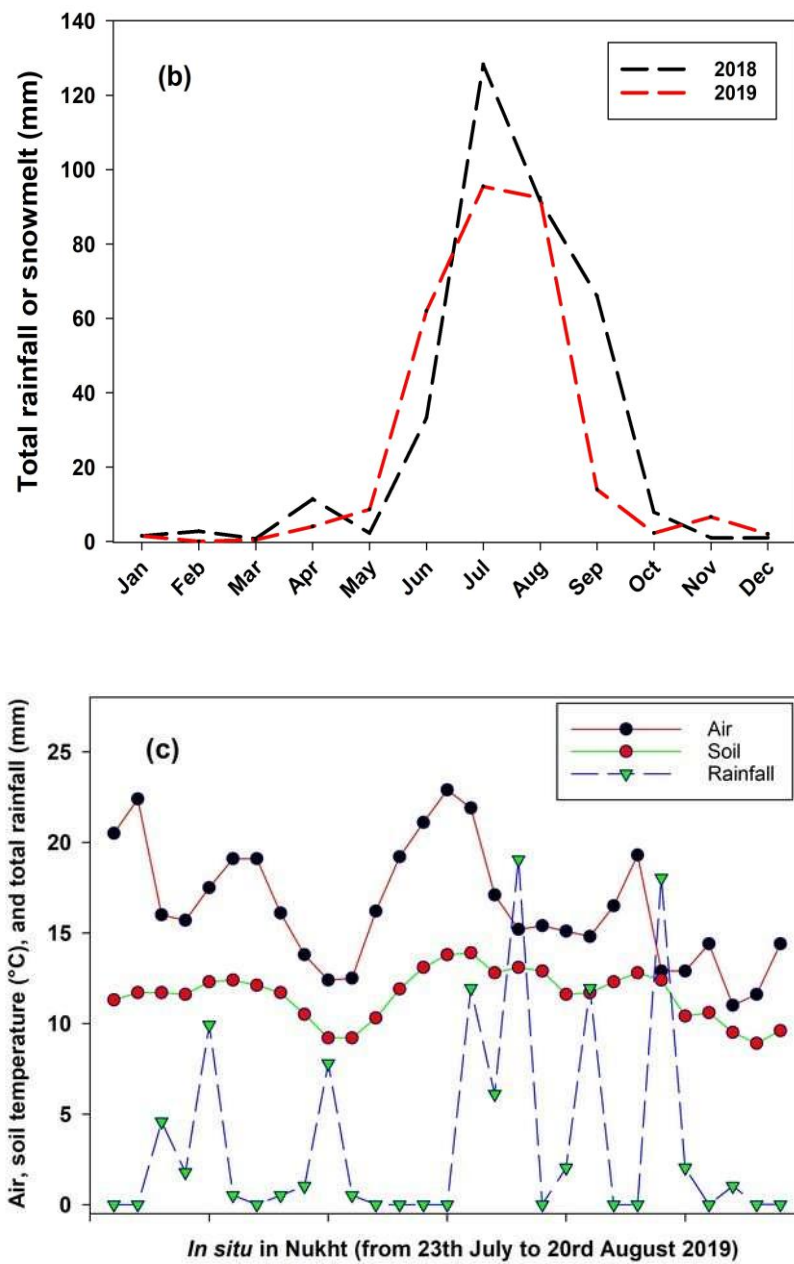


Figure 3: (a) Average air temperature and monthly means rainfall and snowmelt for the Nukht and Batsumber sites in 2019. For Nukht, the data are from weather station ZMUB 44290 at the International airport (www.en.tutiempo.net). The average air temperature data for Batsumber was obtained from the Forest Research Training Center in Udleg, Mongolia. (b) Mean annual precipitation. Average daily soil temperature at 10 cm soil depth at Nukht and the daily air temperature measurements at weather station ZMUB 44290 (c).

5.1.2 Water content

The water content (WC) in % water based on soil dry weight was determined for all sampling dates at both sites Figure 4. During period measurement, WC was ranged from a minimum of 23.7% in June and a maximum of 66.3 Vol.% in March. In March and early October, the maximum soil water content was higher than in June. *Betula platyphylla* shows a relative constancy in comparison to conifer species.

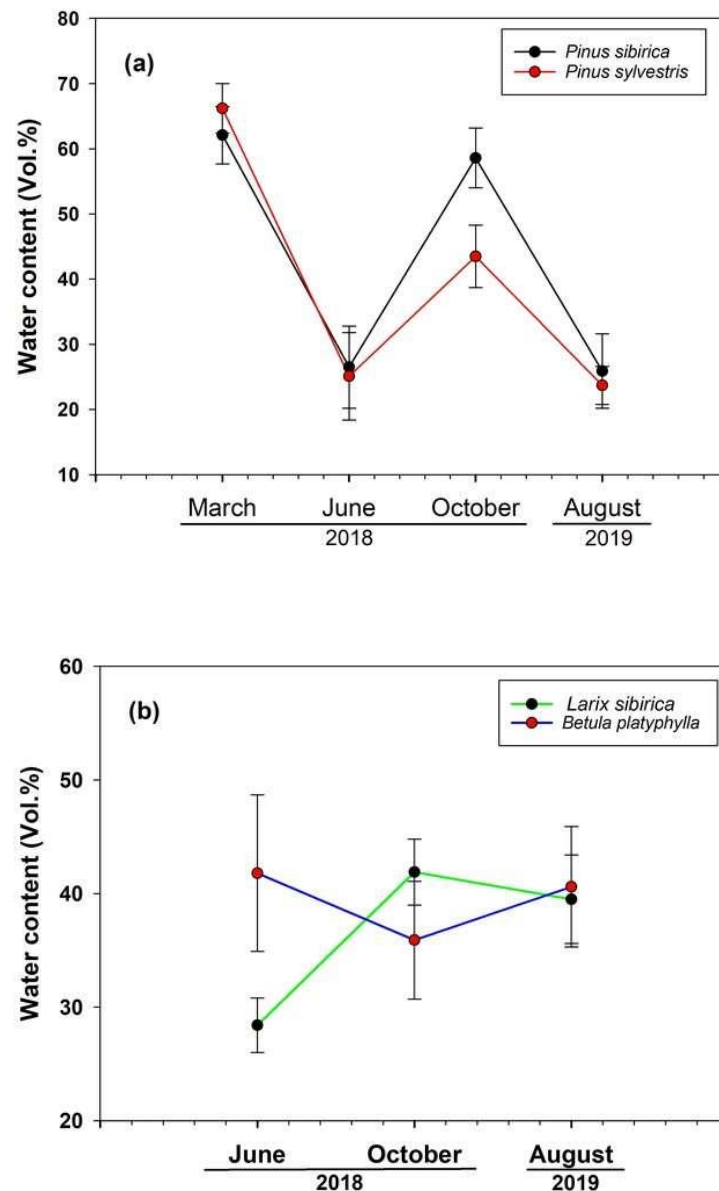


Figure 4: Water content of the soils (0 -10 cm) under the different tree species at Nuhkt (a) (*Pinus sibirica* and *Pinus sylvestris*) and Batsumber (b) (*Larix sibirica* and *Betula platyphylla*). Shown are mean and standard error ($P \leq 0.05$).

5.1.3 pH-value

The pH-value of the soils ranged from 4.81 to 6.47. The pH was highest under *Larix sibirica* and was significantly different to the pH of the soil under *Pinus sylvestris*. The following Table 3 shows the average pH values of the locations for two years. The comparison of the results from both locations showed that there is a significant difference between *Pinus sibirica* and *Pinus sylvestris* at the Nukht ($P \leq 0.05$). In the case of Batsumber, no significant difference was found between 2 tree species and also between the two years (Table 3). Between the sites Nukht and Batsumber, there was a statistical difference in the pH values ($P \leq 0.05$).

5.1.4 C and N and C/N-ratio

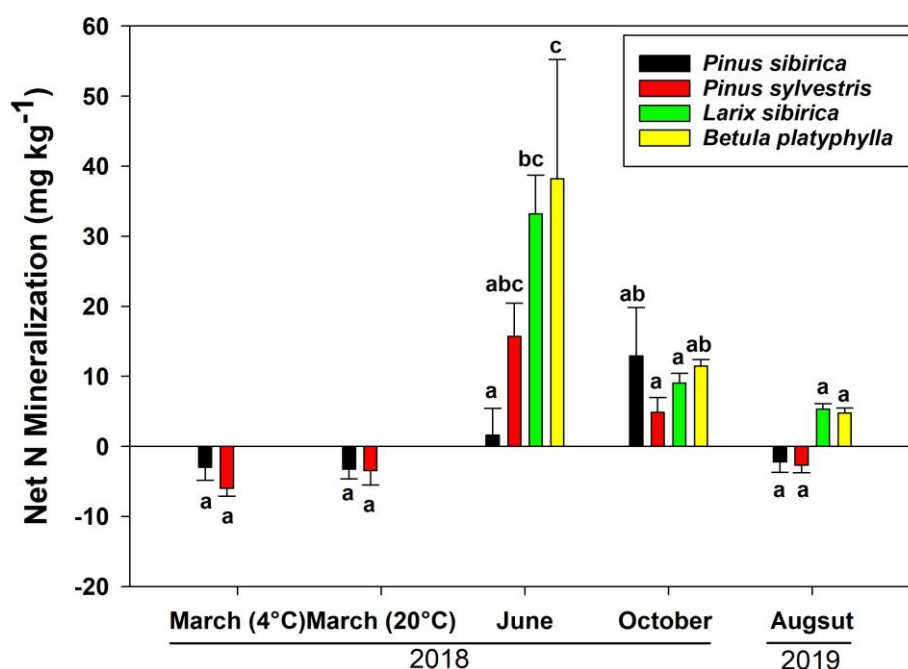
In both Nukht and Batsumber, the percentage C was not significantly different between tree species and years ($P \leq 0.05$). For three species of *Pinus sylvestris*, *Larix sibirica* and *Betula platyphylla*, the percentage N content was lower in 2019 compared to 2018. The C/N-ratio in the soil varied between 16.3 and 20.2 (Table 3). For the C/N-ratio, there was no significant difference for tree species and years ($P \leq 0.05$).

Table 3: Soil properties were taken from under *Pinus sibirica* and *Pinus sylvestris* at Nukht in Mt. Bogd Khan (1514-1600 m) and under *Larix sibirica* and *Betula platyphylla* at Batsumber (1300-1400 m), Mongolia. Values are means \pm SE. Data points within a parameter that are not followed by the same letter (abc) are significantly different ($P \leq 0.05$) between species or years.

	Year	<i>Pinus sibirica</i>	<i>Pinus sylvestris</i>	<i>Larix sibirica</i>	<i>Betula platyphylla</i>
Soil parameters		Nukht		Batsumber	
Soil pH (H ₂ O)	2018	6.1 \pm 0.05abc	5.7 \pm 0.16a	6.4 \pm 0.06c	6.3 \pm 0.11bc
	2019	6.0 \pm 0.14abc	5.8 \pm 0.11ab	6.5 \pm 0.10c	6.2 \pm 0.05bc
Soil pH (CaCl ₂)	2018	5.1 \pm 0.08ab	4.8 \pm 0.14a	5.2 \pm 0.13ab	5.5 \pm 0.12b
	2019	5.2 \pm 0.13ab	5.1 \pm 0.16ab	5.6 \pm 0.05b	5.3 \pm 0.13ab
C%	2018	21.9 \pm 1.05a	21.2 \pm 2.92a	25.1 \pm 0.75a	24.5 \pm 1.76a
	2019	20.3 \pm 3.07a	18.2 \pm 3.49a	15.6 \pm 3.56a	17.5 \pm 3.97a
N%	2018	1.1 \pm 0.04ab	1.1 \pm 0.12ab	1.5 \pm 0.05b	1.4 \pm 0.05ab
	2019	1.0 \pm 0.12ab	0.9 \pm 0.14a	0.9 \pm 0.19a	0.9 \pm 0.14a
C/N-ratio	2018	19.6 \pm 0.78a	19.4 \pm 0.82a	16.3 \pm 0.67a	18.1 \pm 0.91a
	2019	19.9 \pm 0.95a	20.2 \pm 0.74a	18.0 \pm 0.48a	19.2 \pm 1.82a

5.1.5 N-mineralization

Net N-mineralization was measured on soils taken in early spring, the growing season and beginning of winter in 2018 and the growing season 2019. Soil net N-mineralization measured after incubation at 20 °C. The NH_4^+ mineralization ranged over the observation period from a minimum of -6.86 in March (*Pinus sibirica*) to a maximum of 33.00 mg kg^{-1} in June (*Larix sibirica*). Very low or negative NH_4^+ rates were determined during the Nukht in early March and mid-August. In June the ammonification rate in soils from *Pinus sylvestris* at Nukht and, especially under *Larix sibirica*, *Betula platyphylla* stands increased significantly. For *Larix sibirica* and *Betula platyphylla* generally, positive values were observed, *Betula platyphylla* and *Pinus sibirica* at Nukht showed positive and negative values. The laboratory data show no significant difference at 4 °C between 20 °C degrees in March (Fig. 5). NO_3^- mineralization ranged from a minimum of 0.04 in June (*Pinus sibirica*) to a maximum of 5.78 mg kg^{-1} in October (*Larix sibirica*). In October in all vegetation types and both sites, higher NO_3^- mineralization rates were shown than in the other months. *Betula platyphylla* of October showed a significantly NO_3^- rate than other different vegetation species. Nukht of June was found a significantly higher NO_3^- rate between different Batsumber. At Batsumber, in August under *Larix sibirica*, *Betula platyphylla* there was also a difference in NO_3^- mineralization between the *Pinus sibirica* and *Pinus sylvestris* (Fig. 5).



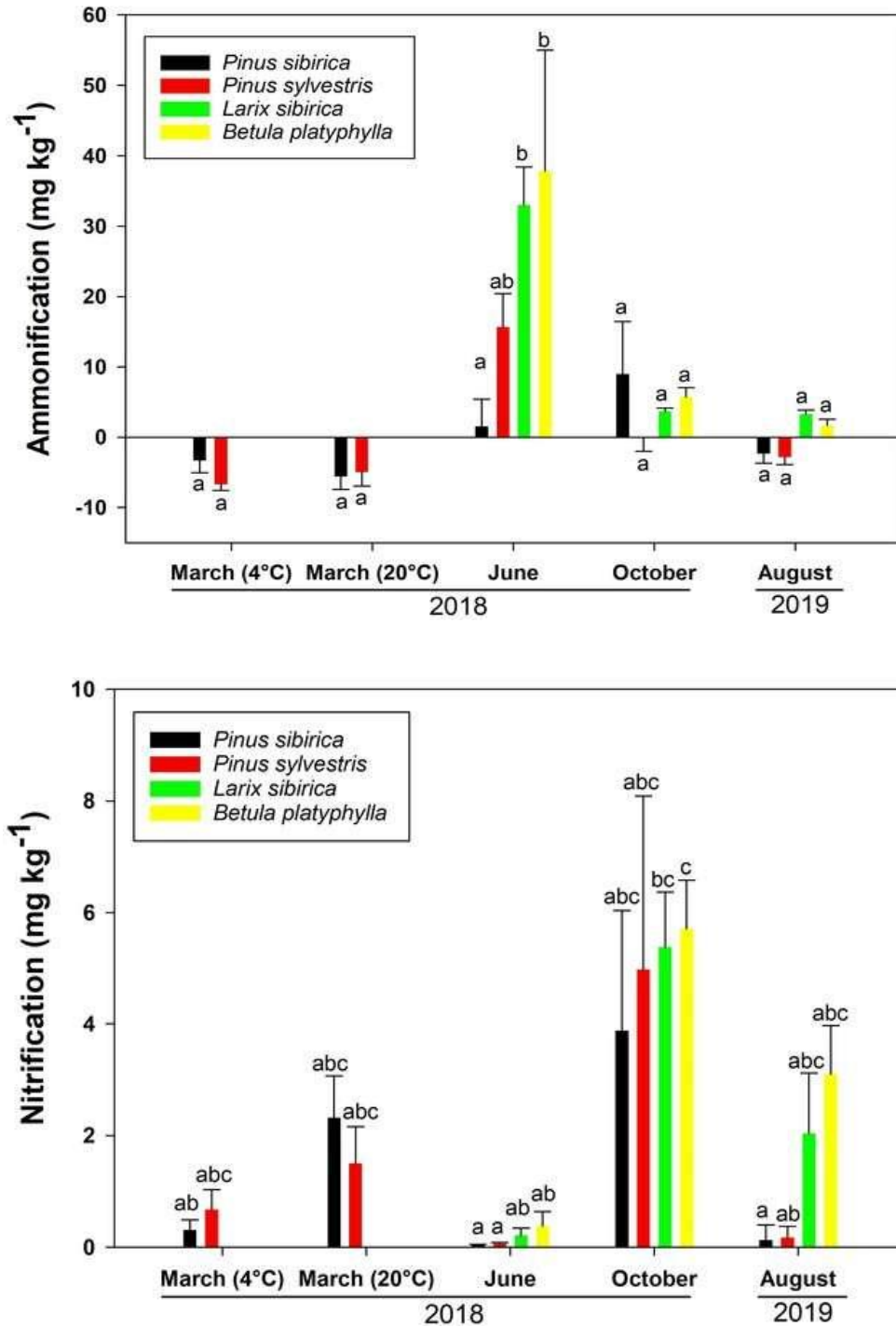
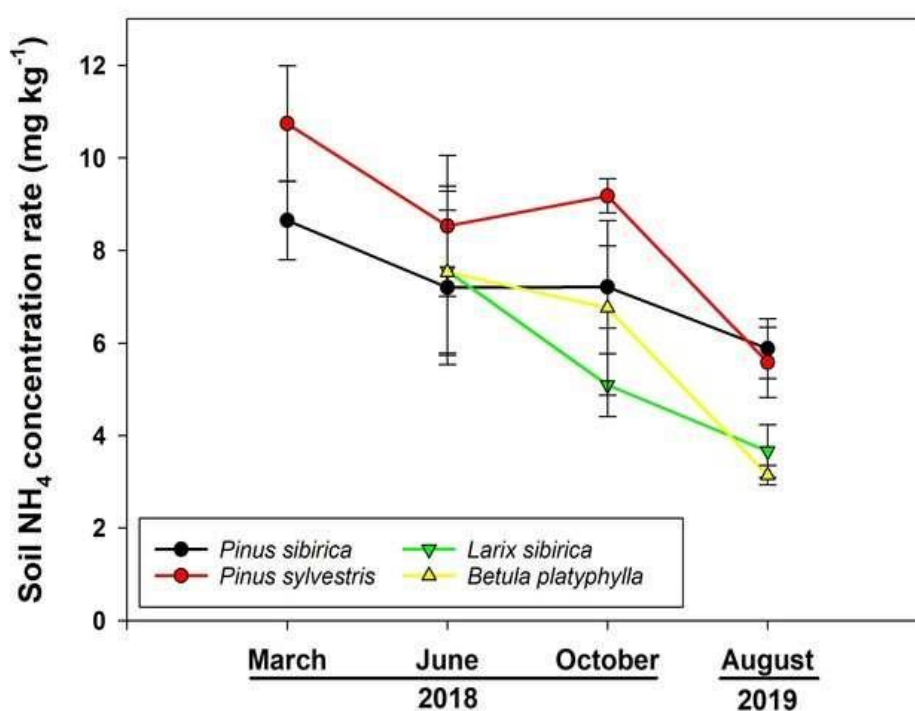


Figure 5: Net ammonification and nitrification measured after incubation at 4 and 20 °C for 30 days of different soil samples taken from under *Pinus sibirica*, *Pinus sylvestris* at Nukht and under *Larix sibirica*, *Betula platyphylla* at Batsumber. Values that are not followed by the same letter are significantly different ($P \leq 0.05$) between species and months in 4 phases.

5.1.6 Soil N availability

For both sites, the extractable NH_4^+ is shown in Figure 6. The NH_4^+ concentrations ranged from a minimum of 2.30 in August (*Pinus sibirica*) to a maximum of 10.7 mg kg^{-1} in March (*Pinus sylvestris*) over the observation period differences between months and years were observed. Concentration in March was higher than in the other months. In general, from March 2018 to August 2019, the levels of extractable gradient NH_4^+ decreased at both sites.

The NO_3^- values of extractable ranged from 0.79 to 7.20 mg kg^{-1} at Nukht the values were similar in March, June and also August, but were significantly higher for *Pinus sylvestris* in October not even recorded differently at sites, similar values in March, and the low value was in March and *Larix sibirica* there significant changes in concentration over the whole investigated period. The values had increased significantly.



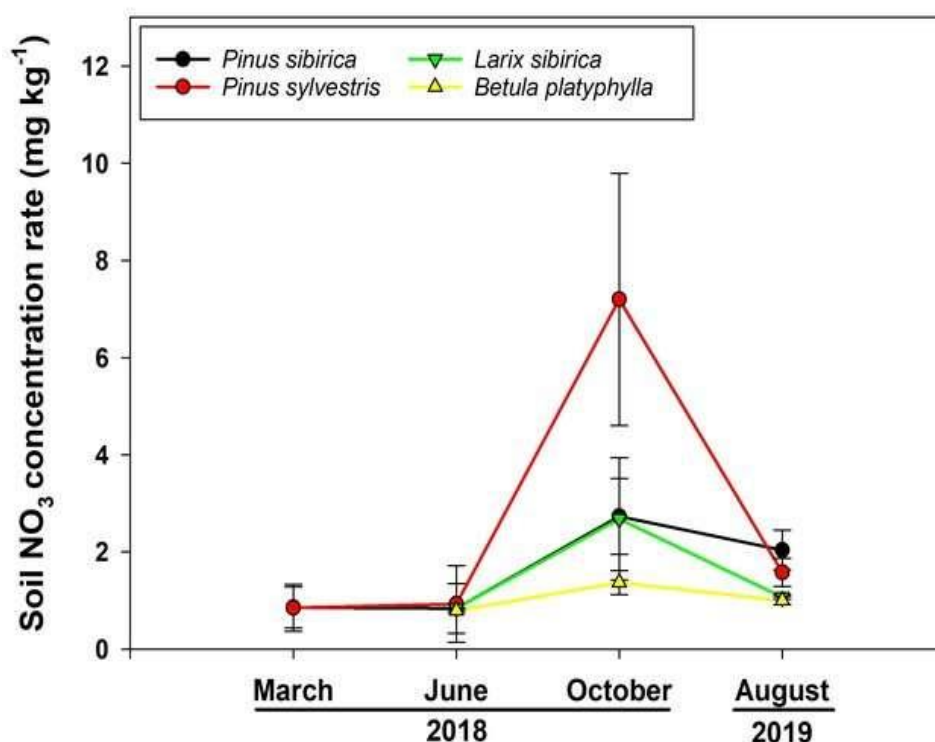


Figure 6: Soil N availability measured from under *Pinus sibirica*, and *Pinus sylvestris* at Nukht and under *Larix sibirica*, *Betula platyphylla* at Batsumber. Values that are not followed by the same letter are significantly different ($P \leq 0.05$) between species and months in 4 phases.

5.1.7 DOC, DON and TN

The seasonal pattern of DOC concentrations ranged from a minimum of 11.6 in August (*Betula platyphylla*) to a maximum of 263.1 mg kg⁻¹ in June (*Pinus sylvestris*) over the observation period (Table 4). In March was significant differences between *Pinus sibirica*, *Pinus sylvestris* ($P \leq 0.05$). From June until August 2019, the DOC concentrations were more similar. However, in October (*Pinus sibirica*) and August 2019 (*Betula platyphylla*) significant differences were found.

DON concentrations ranged from a minimum of 16.98 in June (*Betula platyphylla*) to a maximum of 124.4 mg kg⁻¹ in October (*Betula platyphylla*). The DON concentrations Batsumber showed an increase, and significantly higher differences were found in

October for *Larix sibirica*, and *Betula platyphylla* (Table 4). Otherwise, the following Table 4 shows for all treatments similar concentrations in March, June and August 2019.

TN concentrations ranged from a minimum of 27.85 in June (*Larix sibirica*) to a maximum of 132.6 mg kg⁻¹ in October (*Betula platyphylla*). Significant differences were found in August, and early October at Batsumber.

Table 4: The DOC concentration, DON and TN soils were taken from under *Pinus sibirica*, and *Pinus sylvestris* at Nukht and under *Larix sibirica*, *Betula platyphylla* at Batsumber, Mongolia. Values are means \pm SE. Values not followed by the same letter are significantly different ($P \leq 0.05$) between species and months in 4 phases.

Soil parameters	Month	<i>Pinus sibirica</i>	<i>Pinus sylvestris</i>	<i>Larix sibirica</i>	<i>Betula platyphylla</i>
		Nukht		Batsumber	
DOC (mg kg ⁻¹)	Mar (2018)	249.8 \pm 13.6ab	263.1 \pm 14.4b		
	Jun (2018)	172.4 \pm 33.2ab	195.5 \pm 48.2ab	194.5 \pm 21.6ab	145.8 \pm 3.7ab
	Oct (2018)	100.9 \pm 36.7a	148.7 \pm 31.4ab	215.8 \pm 49.9ab	203.9 \pm 23.9ab
	Aug (2019)	153.4 \pm 21.2ab	113.6 \pm 19.1ab	162.2 \pm 29.1ab	111.6 \pm 13.1a
	Mar (2018)	30.6 \pm 2.4a	27.8 \pm 3.5a		
DON (mg kg ⁻¹)	Jun (2018)	47.3 \pm 9.1a	30.2 \pm 7.2a	19.4 \pm 2.9a	16.9 \pm 1.9a
	Oct (2018)	39.3 \pm 16.5a	31.44 \pm 4.8a	103.9 \pm 14.2b	124.4 \pm 9.4b
	Aug (2019)	58.9 \pm 13.5a	42.11 \pm 10.0a	30.7 \pm 5.9a	24.2 \pm 2.6a
	Mar (2018)	40.1 \pm 2.7a	39.26 \pm 3.4a		
	Jun (2018)	55.3 \pm 9.3a	39.7 \pm 7.4a	27.8 \pm 3.7a	25.3 \pm 1.6a
TN (mg kg ⁻¹)	Oct (2018)	49.3 \pm 2.7a	47.8 \pm 4.4a	111.7 \pm 13.3b	132.6 \pm 9.9c
	Aug (2019)	68.2 \pm 14.3c	51.4 \pm 9.7ab	35.5 \pm 6.5a	28.4 \pm 2.5a

5.1.8 Soil *in situ* N-mineralization

Figure 7 shows no significant difference in ammonification and nitrification rates between the tree species or at the different elevations for both the *in situ* incubation (a) and a laboratory incubation (b). Between the *in situ* and laboratory incubation no significant differences were found for both ammonification and nitrification.

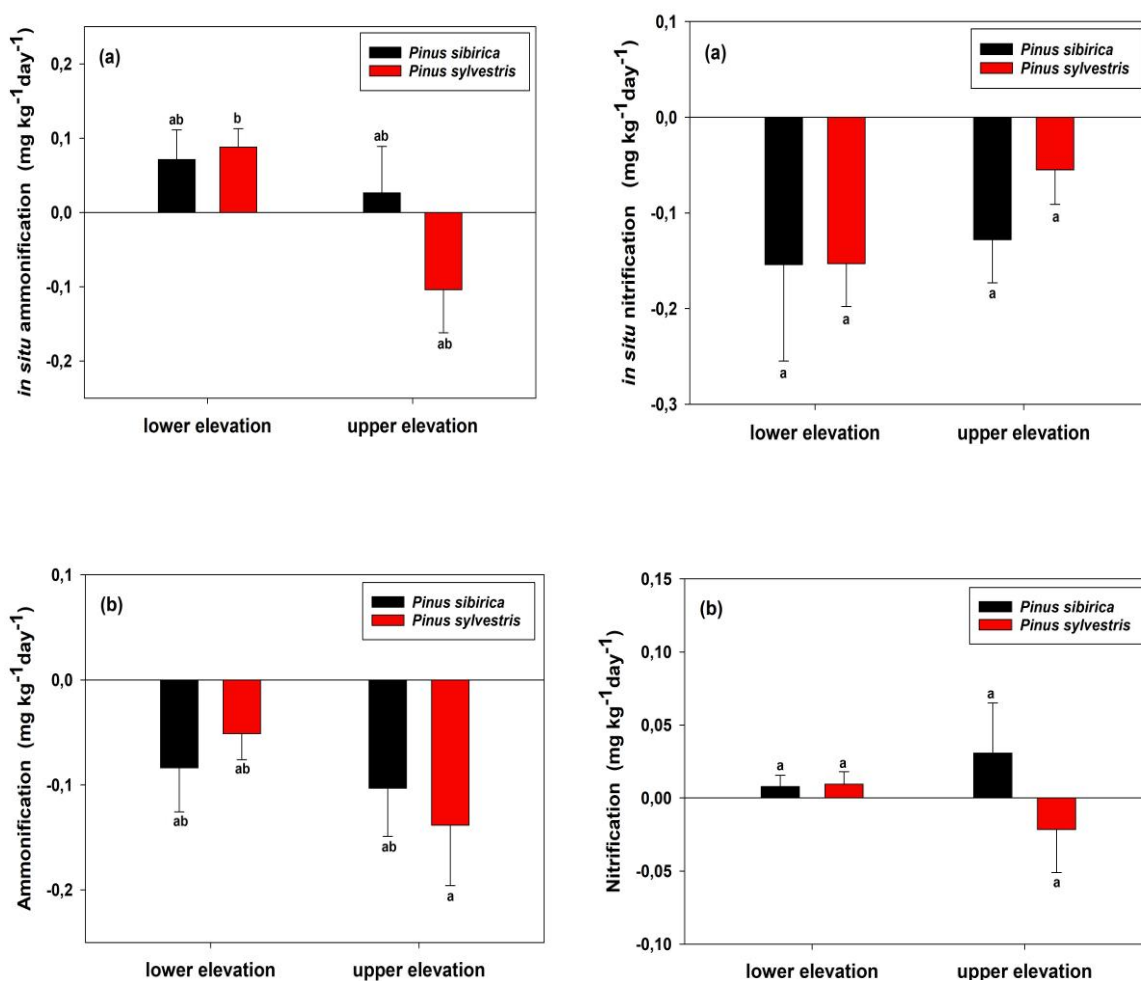


Figure 7: Net ammonification rates (upper panels) and nitrification rates (lower panels) of soil from under of *Pinus sibirica*, *Pinus sylvestris* at a lower elevation (1514-1540 m), and an upper elevation (1600 m) from 23rd July to 20th August 2019. Values that are not followed by the same letter are significantly different (P ≤ 0.05) between species and elevations. (a) *in situ* incubation and (b) laboratory incubation.

5.1.9 Phosphorus

There were no significant differences in P concentrations, but for *Pinus sylvestris*, *Betula platyphylla* and *Larix sibirica* the P content ranges from 46.5 to 54.7mg kg, the lowest being 32.9 mg kg *Pinus sibirica*.

Table 5: NH₄F extractable P of soil taken from under *Pinus sibirica*, *Pinus sylvestris* at Nukht, and *Betula platyphylla*, *Larix sibirica* at Batsumber (August 2019). Values are means \pm SE. Values not followed by the same letter are significantly different ($P \leq 0.05$) between species.

	Nukht		Batsumber	
	<i>Pinus sibirica</i>	<i>Pinus sylvestris</i>	<i>Larix sibirica</i>	<i>Betula platyphylla</i>
Phosphorus (mg kg)	32.9 \pm 7.1a	54.7 \pm 17.9a	51.4 \pm 6.0a	46.8 \pm 11.1a

5.2 Biotic parameters

5.2.1 Ectomycorrhiza

Ectomycorrhizas were extracted from the surface soil horizons of two forest sites. Root tips for every plot were determined. In a total of 1754 ectomycorrhizal root tips were sorted, 506 from *Pinus sibirica* and 7 different morphotypes were found (Fig. 8), in *Pinus sylvestris* (Fig. 9) 291 morphotypes were found in 6 tips. For *Larix sibirica* 90 tips were assessed, and 4 morphotypes were found (Fig. 11), and for *Betula platyphylla* 858 EM in fine root tips were counted and 6 morphotypes were found (Fig. 10). The various morphotype samples were stored in the cold room at -20 °C.

5.2.2 Microbial biomass C and N

Microbial biomass carbon (MB-C) ranged between 99.0 and 101.2 mg kg⁻¹ for *Pinus sibirica*, *Pinus sylvestris* in March. *Pinus sylvestris* showed a significant difference in MB-C. Microbial biomass nitrogen (MB-N) ranged between 10.0 and 10.1 mg kg⁻¹, and the values were quite similar. There was not a significant difference between the two tree species ($P \leq 0.05$).

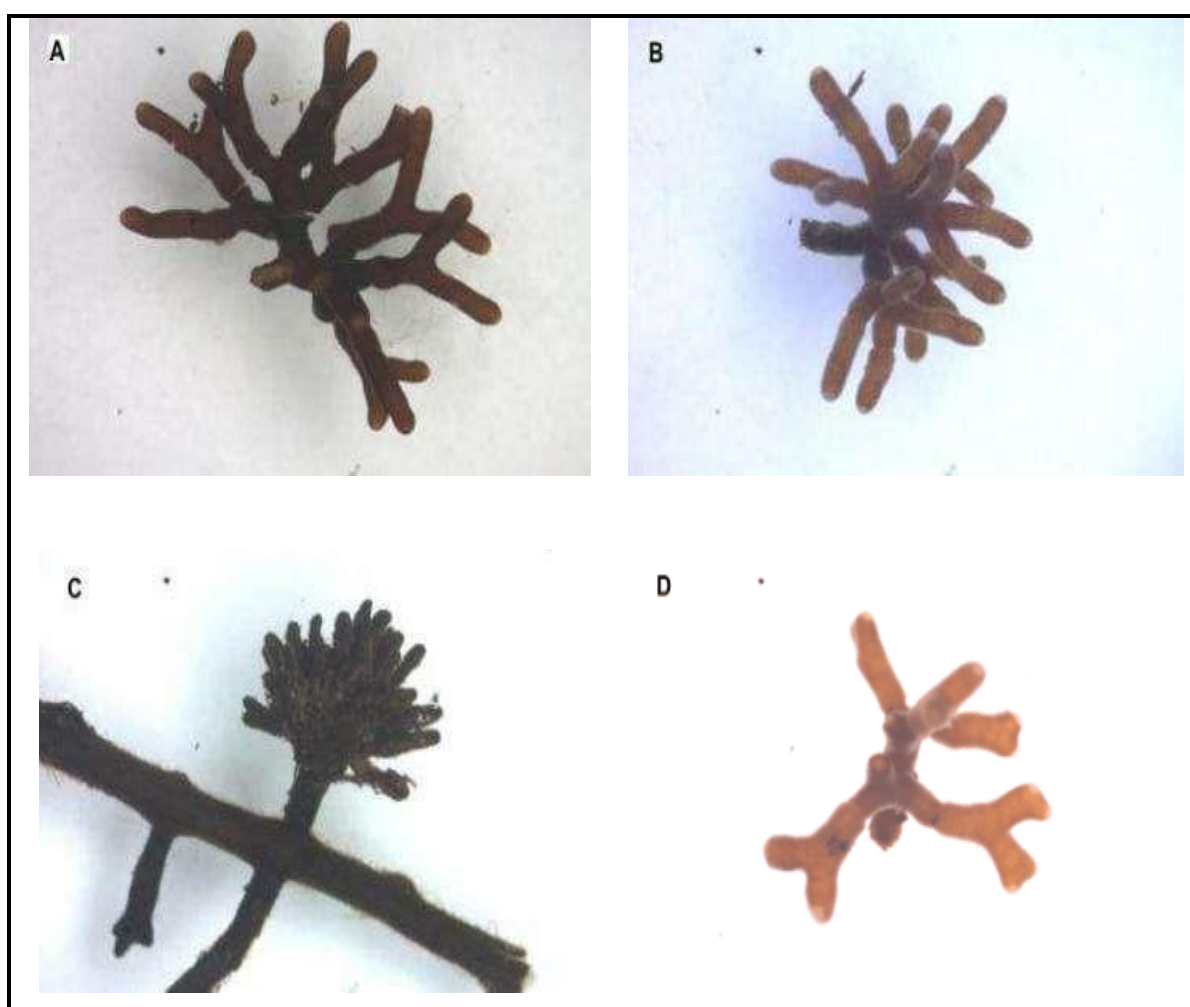
Table 6: Microbial biomass of soil taken from under *Pinus sibirica*, *Pinus sylvestris* at Nukht (Microbial biomass had performed only in March 2018). Values are means \pm SE.

Values not followed by the same letter are significantly different ($P \leq 0.05$) between species.

	<i>Pinus sibirica</i>		<i>Pinus sylvestris</i>	
	MB-C	MB-N	MB-C	MB-N
Microbial biomass (mg kg)	99.0±8.8a	10.0±1.3a	101.2±10.7b	10.1±1.6a

Photo and description of EM morphotypes of tree species.

EM morphotypes of *Pinus sibirica*:



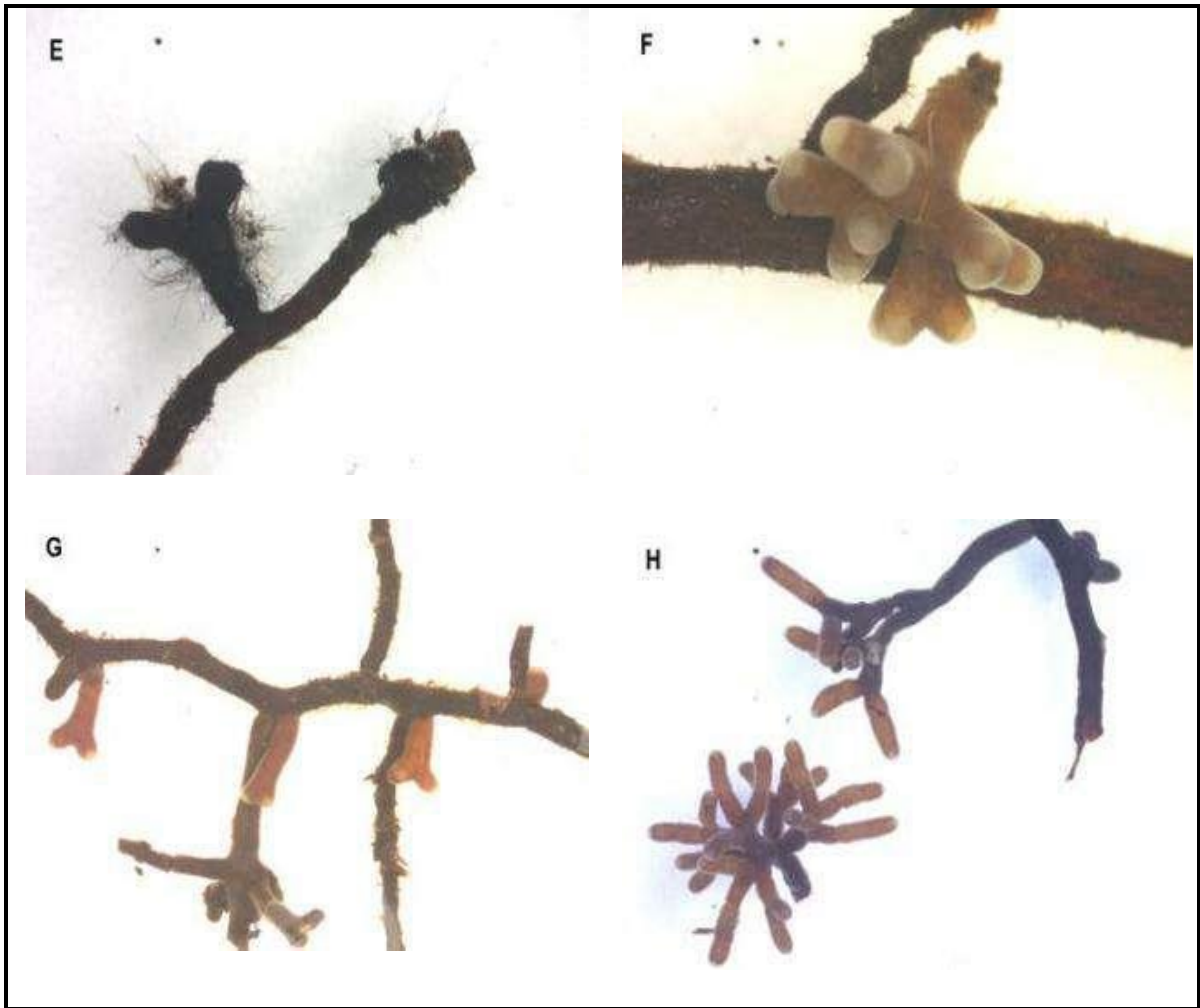


Figure 8: EM morphotypes on *Pinus sibirica* of the Nukht. (A) branched, branched ends dark brown, tips brown-yellowish (84), (B) branched, branched: yellowish-brown (107), (C) many small branching at one branch, branches black (4), (D) two branches, light yellowish (94), (E) two branches, whole branches black with hairy (58), (F) branched, branching light yellow and whitish tips (17), (G) branched and light yellow (7), (H) many light brown, yellowish branches (135).

EM morphotypes of *Pinus sylvestris*:

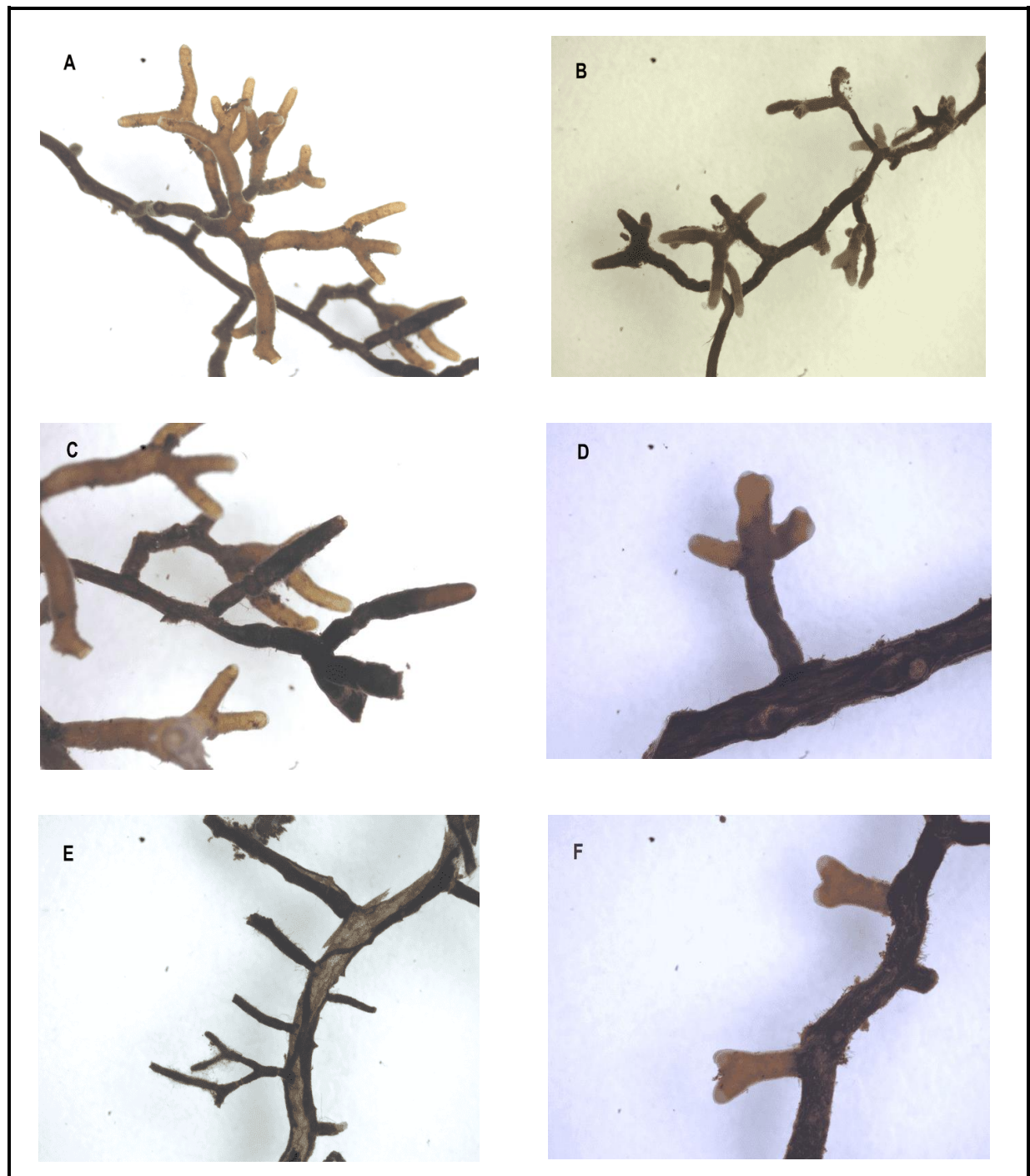


Figure 9: Description of the EM morphotypes and their identified EM fungal partners on *Pinus sylvestris* of the Nukht. (A) branched, yellowish, branches white tips (96), (B) branched, mixed with black, white and brown (108), (C) branched, branches black and yellowish, (D) three branches, brown-yellowish and whitish tips (34), (E) two and one branches, branches black with hairy (40), (F) brown-yellowish and whitish tips (13).

EM morphotypes of *Betula platyphylla*:

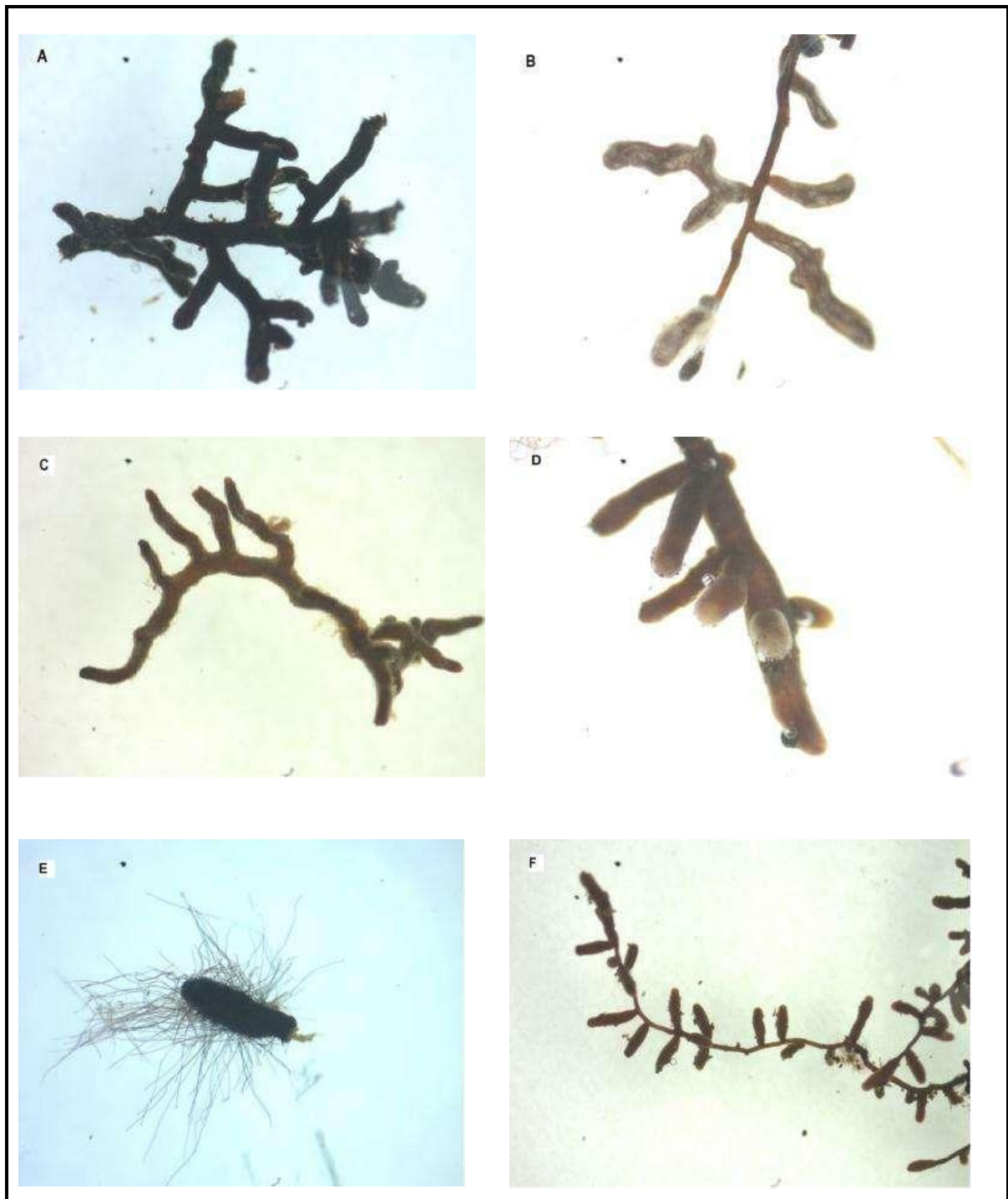


Figure 10: EM morphotypes on *Betula platyphylla* of the Batsummer. (A) branched and branched black tips not hairy (98), (B) branches: ochre, yellowish-brown, tips brownish and cottony (130), (C) branched yellowish-brown (238), (D) branched and branches white tips, yellowish-brown (34), (E) unbranched and long-haired (202), (F) many small branches, dark brown (156).

EM morphotypes of *Larix sibirica* :

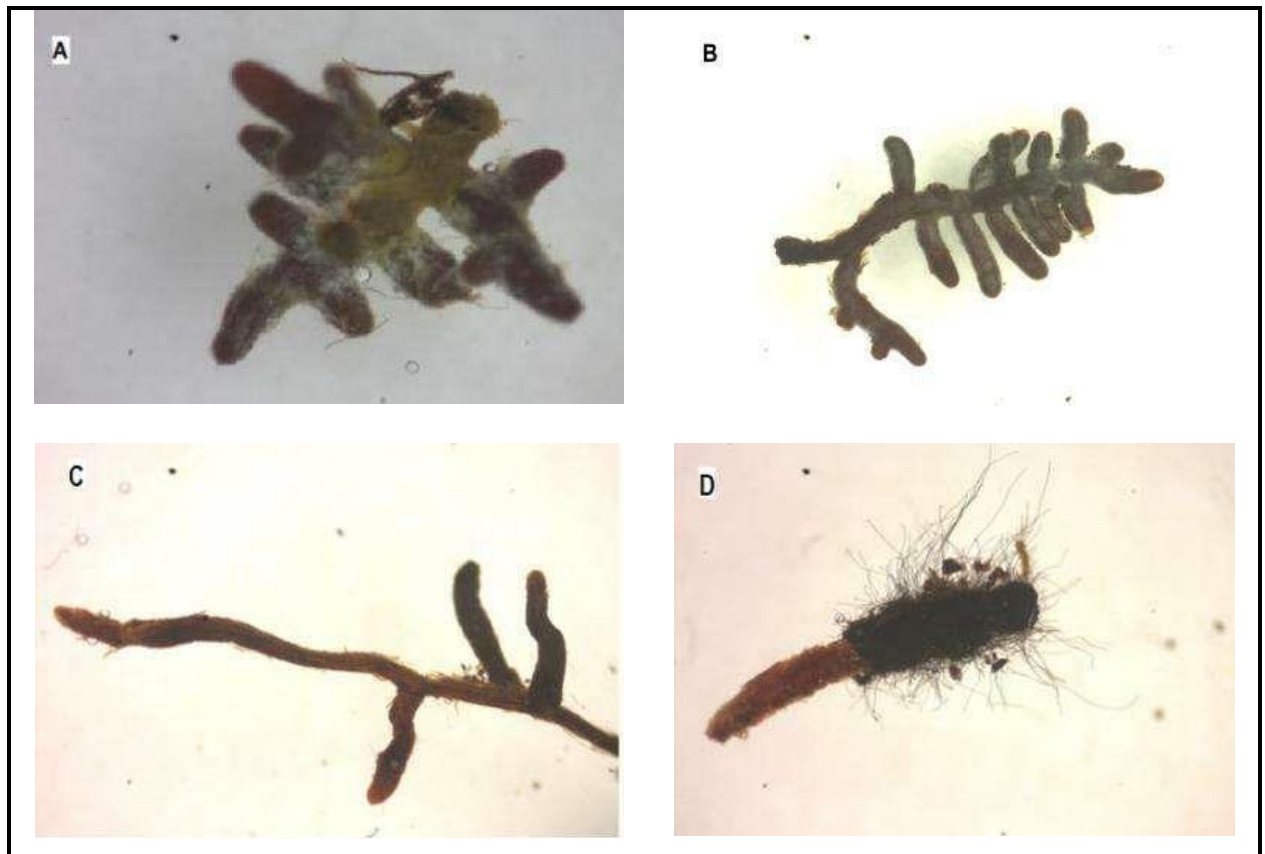


Figure 11: EM morphotypes on *Larix sibirica* of the Batsummer. (A) small branched, tips yellowish (13), (B) branched, brownish and cottony (17), (C) branched, dark brown (24), (D) unbranched, black hairy tips (36).

6 Discussion

6.1 Soil abiotic parameters

The N-mineralization and nitrification rates generally depend on several factors, including soil temperature and moisture conditions (Gadgil and Gadgil., 1978). Soil temperature has enormous ecological effects through evaporation, transpiration, decomposition of organic matter, and permafrost thawing. In forest ecosystems, soil temperature regulates microbial transformation of N, and other nutrients (Plymale et al., 1987). The average monthly soil temperature over the last 4 years has been between -13 °C and +10 °C (Otgonsuren et al., 2020). Changes in temperature influence the microbiological activity in the soil and thereby denitrification and nitrification. Increasing the soil temperature improves root growth and increases metabolic activity (Repo et al., 2004). Several studies have shown that the mineralization rate depends on soil temperature. The soil temperature at Nukht showed a wide range. During the growing season, the soil temperature was between 9.8 and 11.0 °C, which is similar to tree line forest in Austria (Godbold unpublished data). However, at Nukht, the soil temperature was below 0 °C for nearly 6 months.

Soil pH directly influences biodiversity and plant growth due to the pH-dependent absorption capacity of micro and macro elements, soil formation, transformation and regeneration, and ion exchange (Blum., 2007). The analysis of the pH value was performed in both H₂O and CaCl₂ solution. Usually, the pH measurement leads CaCl₂ to lower than those measured in H₂O (Blume et al., 2010). Several studies have shown that soil pH increases, along with an increase in total soil N and decrease C/N-ratio (Giesler et al., 1998; Högberg et al., 1990). Nukht and Batsumber, there was a statistical difference in pH values between the sites, and the result clearly showed a strong influence of pH on mineralization. Soil moisture was highest at Nukht, and the pH value is lowest (Table 3, Fig. 4a).

The C of the soil was constant between the two years, no significant differences were found between the species or the sites. Similarly, for N no significant differences were found between the species or the sites, but differences were found between the years. The lower N values in 2019, might be due to that the soil samples contained different amounts of the soil layers. Forest floor components, such as deadwood litter or biomass from herb projectiles, presumably affect the decay or the C/N-ratio.

The ability of the WC of the soil plays a vital role in ideal conditions for plant growth. Soil WC regulated microbial processes and ecological interactions involved in nutrient cycling.

It influences the rate and pathways for microbial transformation of nitrogen (Amador et al. 2005). The soil water content over the observation period of Nukht and Batsumber site is shown in Figure 4. General environment conditions in summer were quite dry, and there was scarce rainfall. March and early October showed that the maximum soil WC was higher than in June and August, because, by the end of March, the ground was still frozen. Besides, rainfall increased from July to the end of September and composed most of the annual precipitation (Fig. 3b). Notably, a low value was shown for both locations in June. The WC of the soil under *Betula platyphylla* shows relatively constant in comparison to the conifer species. The cause was a period of dry weather before the sampling procedure was performed (10.06.2018). Moreover, all plants do not wilt at the same water content (Osman., 2013). The soil WC content depends on many reasons. For example, varies enormously depending on these soil properties, the plant characteristics, including leaf area, stomatal density, leaf resistance, rooting density and rooting depth. WC contents correlate with received precipitation or ground thawing and outputs that depend on vegetation demand and infiltration. Ammonification in the soil is less sensitive to water content than nitrification (Paul and Clark., 1996). In October, a higher nitrification rate was shown, for Nukht this corresponded to a higher soil moisture content. The result showed a correlation between WC and nitrate.

6.2 Net N-mineralization

The nitrogen dynamics of the soils in the current study differ significantly between the two forests, especially in June, there was a significant difference between the tree species. Since all stands are in relatively similar climates, this difference is likely due to the influence of tree species. Moreover, herbivores influence primary productivity by accelerating the nitrogen mineralisation rate (Semmartin and Oesterheld., 2001). There is also a nomadic lifestyle closer to the Batsumber study area, with livestock grazing in the area. Perhaps N-mineralization could be influenced.

The N-mineralization and nitrification rates are generally dependent on several factors, including soil temperature and moisture conditions (Gadgil and Gadgil., 1978). The higher rates of mineralization can be explained by the interactions between microbial abundance, soil moisture and substrate availability. Several studies have identified a positive correlation between moisture and net N-mineralization rate (Kowalenko and Cameron., 1976; McMillan et al., 2007). Low nitrification rates in boreal soils have been associated with low pH and stand type (Ste-Marie and Paré., 1999). The Nukht site has a lower

average soil pH compared to the soil pH values at Batsumber, and there is also a tendency to lower N-mineralization rates at Nukht.

The increased NH_4^+ concentrations suggest that ammonification rates increase during the growing season in June, mainly at Batsumber. There were few significant differences in the amounts of NH_4^+ in the soil or the rates between the sites or tree species, however, in June, the ammonification rate of *Larix sibirica*, and *Betula platyphylla* increased significantly. When the ammonification rates were high in June 2018, nitrification rate was low, especially in the soil of *Pinus sibirica* and *Pinus sylvestris*, which can be shown in Figure 5. Until mid-June in particular, the soil at both locations was dry with little precipitation during the summer (Fig. 3a, b). Ammonification depends on seasonal dynamics and soil properties, such as soil water content (Holik et al., 2017). Figure 5 generally shows low or negative concentrations of ammonification as the water content increased. In June, the soil under *Larix sibirica* and *Betula platyphylla* had the highest NH_4^+ concentration. According to Allison et al. (2009), N availability in boreal soils increases due to N deposition, N-mineralization increases with global warming. However, the laboratory data from March showed no significant temperature effect on ammonification and nitrification (Fig. 5). However, the measurements were carried out in laboratory incubation under control temperatures at 4 and 20 °C. At both temperatures, the net rate of ammonification was negative, suggesting an immobilization of NH_4^+ in the microbial biomass. The low but positive levels of nitrification suggest however that ammonification must have taken place as nitrification is dependent upon the presence of NH_4^+ . In both incubations, there was no significant difference in soil NO_3^- rates under *Pinus sibirica*, *Pinus sylvestris* lower elevation and upper elevation. However, significantly different NH_4^+ rates were found under *Pinus sylvestris* in a laboratory and *in situ* incubation compared to elevation. The negative NH_4^+ rates of measured in the laboratory incubation of *Pinus sibirica*, *Pinus sylvestris* at both elevations is an indication of microbial immobilization in these soils. Temperature fluctuations and soil moisture may have contributed to the N-mineralization of the *in-situ* incubation period (Knoepp and Swank., 2002). The air temperature varied between 11 and 22.9 °C during the incubation period *in situ*. The mean air temperature was 16.4 °C, and soil means temperature was 11.5 °C (Fig. 3c, Fig. 7, Table 8).

6.3 Soil N availability

Many forest ecosystems are characterized by the growth-limiting availability of N (Rennenberg et al., 2009). Boreal forest ecosystems are generally considered by N-

limited, (Schulze., 2000), where cold temperatures and low decomposition rates dominate the soil. The cause of this limitation is the slow mineralization of soil organic nitrogen (Tamm., 1991). The content of extractable inorganic N from as NH_4^+ and NO_3^- is a balance between N supply from the N-mineralization and N uptake by plants or immobilized by microorganisms (Rennenberg and Dannenmann., 2015). N-mineralization rates are higher during the first thaw cycle (Schimel and Clein., 1996). The N availability rates of NH_4^+ in March was higher than in the other months at Nukht (Fig. 6). This corresponded to the highest DOC value measured at the Nukht site. DOC is known to be released during thawing (Schimel and Clein., 1996). The availability of N at all sites decreased over time. At Batsumber, the soil under *Larix sibirica* and *Betula platyphylla* had compared to Nukht the lowest extractable NH_4^+ and NO_3^- in August. At Batsumber, the C/N-ratio was generally lower overall (Table 3, Fig. 6), which should increase the decomposition and N-mineralization rates. Therefore, due to the lower C/N-ratio, the leaf and possibly root litter of *Larix sibirica* and *Betula platyphylla* compared to *Pinus sibirica* and *Pinus sylvestris*, the litter of *Pinus sibirica* and *Pinus sylvestris* litter should decompose slower (Scott and Binkley., 1997). From March 2018 to August 2019 the levels of extractable gradient NH_4^+ generally decreased at both sites, there was also very little extractable NO_3^- with the exception of *Pinus sylvestris*. Especially at both stands in March, June 2018 and August 2019. The low levels in the summer months could be due to nitrogen uptake of the plants. Low availability of NH_4^+ in March might be due to low mineralization or rapid immobilization. DON plays a vital role in the N cycle, which is the source of both microorganisms and plants. Giesler et al (1998) showed DOC and DON concentrations appear to be related to the forest floor of decomposition and the new litter inputs. Decomposition products should be produced more rapidly at higher temperatures in late summer. The highest DON and TN concentrations appeared in Batsumber and an increase, significant differences were found in *Larix sibirica* and *Betula platyphylla* in October, comparing two stands. Phosphorus (P) is a primary essential nutrient in forest ecosystems. In the boreal forest, there are also P limiting growth (Giesler et al. 1998). The result of P, there were no significant differences between the soils.

6.4 Biotic parameters

Changes in mycorrhizal community composition can have a major impact on the ecosystem, as mycorrhizal associations are essential for most plant species nutrient uptake in a boreal forest (Read., 1991). In forest ecosystems, soil the microbial activity offers excellent potential for recycling and release of available N and microbial competition

with plants for N. In Nukht, 7 morphotypes of EM were determined on *Pinus sibirica*, and none were found on *Pinus sylvestris*. For *Larix sibirica* and *Betula platyphylla*, 4 and 6 different EM morphotypes could be distinguished, respectively (Fig. 10, 11). Otgonsuren et al. (2020) showed that in *Pinus sylvestris*, *Pinus sibirica* that 12 and 14 different EM morphotypes could be distinguished on root tip samples also taken from Nukht. In their study, Otgonsuren et al. (2020) counted a much higher number of root tips over 1000, which explains the difference in the number of morphotypes detected. Tree species is the most important factor influencing the formation of EM communities and the distribution of exploration types of the EM taxa (Rosinger et al. 2018). In agreement with this the morphotypes found on *Pinus sibirica*, *Larix sibirica* and *Betula platyphylla*, appear to be different. However, one morphotype, black with a large amount of mycelium, is very similar to the morphological description of *Cenococcum* (Agerer., 1997) and was found on all 3 tree species. *Cenococcum* is a very commonly occurring mycorrhizal genus (Trappe., 1964). Rosinger et al. (2018) also could show that abiotic factors such as soil properties, N enrichment, temperature, and precipitation also significantly influence EM diversity and community composition. These observations highlight the need for further studies on the EM community in boreal forest ecosystems to identify the N cycle's main functional components. In the soil, transformations of N such as ammonification, immobilisation, and nitrification are microbially mediated processes that control the availability of N for plant uptake (Malchair and Carnol., 2009).

7 Conclusions

The results showed that the N-mineralization rate depends on seasonal climatic conditions, soil properties and forest types. N-mineralization of NO_3^- rates show higher in October than in March, June and August. Soil WC influences the N cycle and N availability in the ecosystem. This work has shown that temperature particularly has a significant influence on nitrogen dynamics. In addition, the example of Batsumber shows that herbivores influence nitrogen mineralisation. The interactions between soils, forest trees, microbial processes, and ground vegetations have not yet been thoroughly researched scientifically, so further research is needed in this area.

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9 Appendix

Table 7: Mean temperature (°C) and total precipitation or snowmelt (mm) of the study period in two years.

Month	2018		2019	
	Temperature (°C)	Total rainfall/or snowmelt (mm)	Temperature (°C)	Total rainfall/or snowmelt (mm)
Jan	-22.30	1.52	-21.5	1.53
Feb	-17.43	2.78	-19.6	0.0
Mar	-2.23	0.76	-5.5	0.5
Apr	4.43	11.43	3.1	4.06
May	13.50	2.28	8.9	8.63
Jun	17.87	33.27	16.8	61.99
Jul	17.77	128.27	18.9	95.5
Aug	17.63	91.69	15	92.4
Sep	8.33	66.04	11.6	13.97
Oct	2.90	7.88	-1.2	2.29
Nov	-8.87	1.02	-12.5	6.61
Dec	-20.17	1.01	-21	2.03

Table 8: Daily air, soil temperature and precipitation during the *in situ* incubation of over a month.

Date	Air Temperature (°C)	Soil Temperature (°C)	Total rainfall (mm)
23/07/2019	20.5	11.3	0.0
24/07/2019	22.4	11.7	0.0
25/07/2019	16.0	11.7	4.57
26/07/2019	15.7	11.6	1.78
27/07/2019	17.5	12.3	9.91
28/07/2019	19.1	12.4	0.51
29/07/2019	19.1	12.1	0.0
30/07/2019	16.1	11.7	0.51
31/07/2019	13.8	10.5	1.02
01/08/2019	12.4	9.2	7.78
02/08/2019	12.5	9.2	0.51
03/08/2019	16.2	10.3	0.0
04/08/2019	19.2	11.9	0.0
05/08/2019	21.1	13.1	0.0
06/08/2019	22.9	13.8	0.0
07/08/2019	21.9	13.9	11.94
08/08/2019	17.1	12.8	6.1
09/08/2019	15.2	13.1	19.05
10/08/2019	15.4	12.9	0.0
11/08/2019	15.1	11.6	2.03
12/08/2019	14.8	11.7	11.94
13/08/2019	16.5	12.3	0.0
14/08/2019	19.3	12.8	0.0
15/08/2019	12.9	12.4	18.03
16/08/2019	12.9	10.4	2.03
17/08/2019	14.4	10.6	0.0
18/08/2019	11.0	9.5	1.02
19/08/2019	11.6	8.9	0.0
20/08/2019	14.4	9.6	0.0

Table 9: The water content of the soils (0 -10 cm) under the different tree species. Shown are mean and standard error ($P \leq 0.05$).

Year	Month	Nukht		Batsumber	
		<i>Pinus sibirica</i>	<i>Pinus sylvestris</i>	<i>Larix sibirica</i>	<i>Betula platyphylla</i>
	Mar	62.1±4.5 ^{cd}	66.3±3.8 ^d		
2018	Jun	26.5±6.3 ^a	25.1±6.8 ^a	28.4±2.4 ^a	41.8±6.9 ^{abc}
	Oct	58.6±4.6 ^{bcd}	43.6±4.9 ^{abcd}	42.0±2.9 ^{abc}	36.0±5.2 ^{ab}
2019	Aug	26.0±5.7 ^a	23.7±2.9 ^a	39.5±3.9 ^{abc}	40.6±5.3 ^{abc}

Table 10: Ammonification and nitrification (4 and 20 °C for 30 days) of soil taken under *Pinus sibirica*, *Pinus sylvestris* at Nukht and from under *Larix sibirica*, *Betula platyphylla* at Batsumber. Shown are mean and standard error ($P \leq 0.05$).

Year	Month	Nukht		Batsumber	
		<i>Pinus sibirica</i>	<i>Pinus sylvestris</i>	<i>Larix sibirica</i>	<i>Betula platyphylla</i>
NH ₄ ⁺ (mg kg ⁻¹)					
2018	Mar (at 4 °C)	-3.29±1.75 ^a	-6.67±0.90 ^a		
	Mar (at 20 °C)	-5.56±1.85 ^a	-4.93±2.02 ^a		
	Jun	1.54±3.87 ^a	15.65±4.48 ^{ab}	33.00±5.42 ^b	37.83±17.16 ^b
	Oct	9.00±7.45 ^a	-0.11±1.89 ^a	3.66±0.46 ^a	5.73±1.34 ^a
2019	Aug	-2.34±1.35 ^a	-2.84±1.03 ^a	3.29±0.59 ^a	1.64±0.90 ^a
NO ₃ ⁻ (mg kg ⁻¹)					
2018	Mar (at 4 °C)	0.31±0.18 ^{ab}	0.68±0.35 ^{abc}		
	Mar (at 20 °C)	2.32±0.75 ^{abc}	1.50±0.66 ^{abc}		
	Jun	0.04±0.01 ^a	0.06±0.54 ^a	0.21±0.13 ^{ab}	0.38±0.52 ^{ab}
	Oct	3.88±2.16 ^{abc}	4.98±3.11 ^{abc}	5.38±0.99 ^{bc}	5.71±0.87 ^c
2019	Aug	0.13±0.27 ^a	0.17±0.20 ^{ab}	2.04±1.08 ^{abc}	3.10±0.87 ^{abc}
Net N-mineralization (mg kg ⁻¹)					
2018	Mar (at 4 °C)	-2.97±1.86 ^a	-5.99±1.11 ^a		
	Mar (at 20 °C)	-3.24±1.40 ^a	-3.43±2.07 ^a		
	Jun	1.58±3.86 ^a	15.71±4.77 ^{abc}	33.22±5.52 ^{bc}	38.22±17.02 ^c
	Oct	12.89±6.98 ^{ab}	4.86±2.12 ^a	9.05±1.38 ^a	11.44±0.96 ^{ab}
2019	Aug	-2.21±1.47 ^a	-2.66±1.09 ^a	5.33±0.78 ^a	4.75±0.73 ^{abc}

Table 11: N availability of Nukht and Batsumber. Shown are mean and standard error ($P \leq 0.05$).

		Nukht		Batsumber	
Year	Month	<i>Pinus sibirica</i>	<i>Pinus sylvestris</i>	<i>Larix sibirica</i>	<i>Betula platyphylla</i>
NH ₄ ⁺ (mg kg ⁻¹)					
2018	Mar	8.65±0.9 ^{abc}	10.74±1.3 ^c		
	Jun	7.2±1.7 ^{abc}	8.5±1.5 ^{abc}	7.6±1.8 ^{abc}	7.5±1.8 ^{abc}
	Oct	7.2±0.9 ^{abc}	9.2±0.4 ^{bc}	5.1±0.7 ^{abc}	6.8±1.9 ^{abc}
2019	Aug	5.9±0.6 ^{abc}	5.6±0.8 ^{abc}	3.7±0.6 ^{ab}	3.1±0.2 ^a
NO ₃ ⁻ (mg kg ⁻¹)					
2018	Mar	0.86±0.04 ^a	0.85±0.04 ^a		
	Jun	0.84±0.05 ^a	0.93±0.07 ^a	0.84±0.02 ^a	0.79±0.06 ^a
	Oct	2.73±0.70 ^a	7.20±2.60 ^b	2.68±1.26 ^a	1.37±0.25 ^a
2019	Aug	2.04±0.41 ^a	1.58±0.29 ^a	1.07±0.04 ^a	1.00±0.09 ^a

Table 12: Net *in situ* ammonification and nitrification of soil from under *Pinus sibirica*, *Pinus sylvestris* at a lower elevation (1514-1540 m), and upper elevation (1600 m) from 23rd July to 20th August 2019 in Nukht. Shown are mean and standard error ($P \leq 0.05$).

	lower elevation		upper elevation	
	<i>Pinus sibirica</i>	<i>Pinus sylvestris</i>	<i>Pinus sibirica</i>	<i>Pinus sylvestris</i>
	<i>in situ</i> incubation			
NH_4^+ (mg kg ⁻¹ day ⁻¹)	0.071±0.03 ^{ab}	0.088±0.02 ^b	0.026±0.06 ^{ab}	-0.104±0.05 ^{ab}
NO_3^- (mg kg ⁻¹ day ⁻¹)	-0.154±0.10 ^a	-0.153±0.04 ^a	-0.128±0.04 ^a	-0.055±0.03 ^a
	laboratory incubation			
	<i>Pinus sibirica</i>	<i>Pinus sylvestris</i>	<i>Pinus sibirica</i>	<i>Pinus sylvestris</i>
NH_4^+ (mg kg ⁻¹ day ⁻¹)	-0.083±0.04 ^{ab}	-0.051±0.02 ^{ab}	-0.103±0.04 ^{ab}	-0.138±0.05 ^a
NO_3^- (mg kg ⁻¹ day ⁻¹)	0.007±0.007 ^a	0.009±0.008 ^a	0.030±0.03 ^a	-0.021±0.02 ^a

10 Photographs

Photography of research areas and laboratory:



Photography of Nukht: Forest stand *Pinus sibirica* and *Pinus sylvestris* Bogd-Khan Mountain (A, E). Soil samples for field incubation (B), and during soil sample collection, 2019 (C), (D) Snowfall in early October 2018. Shrub species in the region *Rosa acicularis* (F).



Photography of Batsumber: View of the research area mixed forest (A). Collection of the soil sample and fieldwork (B) (2019), and the snow-covered research area is beginning of October in the 2018 (C), and *Larix sibirica* (D). A stainless steel core with 10 cm diameter (E).



Photography of laboratory: While measuring the pH values (A), soil preparation for water content (B). C/N samples are saved for repetition if they are mismeasured (C). Fumigation measurement for microbial biomass (D). Preparation for soil sample solution (E), and incubation (30 days) provided at 20 °C. Ectomycorrhiza examination and laboratory work (G, H).