

RESEARCH

Open Access



Role of ectomycorrhizal colonization in enhancement of nutrients for survival of plants collected from mountainous cold stress areas

Aisha Umar^{1*}, Mai Ali Mwaheb², Fuad Ameen³, Fares Almomani⁴, Laurent Dufossé⁵ and Marek Gancarz^{6,7}

Abstract

Background Ectomycorrhizal (ECM and ECM-like) structures associated with plant root systems are a challenge for scientists. The dispersion pattern of roots within the soil profile and the nutritional conditions are both favourable factors to motivate the plants to make ECM associations.

Results This study discusses the colonization of mycorrhizal associations in *Kobresia* and *Polygonum* species including *Polygonum viviparum*, *Kobresia filicina*, *K. myosuroides*, *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens* grown naturally in cold stressed soils of Gilgit-Baltistan (high-altitude alpine Deosai plains), Hazara, Swat, Dir, and Bajaur. Sieved soil batches were exposed to +5 °C (control), -10, -20, -30, -40, -50, -125 °C for 5 h, and selected plants were sown to these soils for 10 weeks under favourable conditions for ECM colonization. Ectomycorrhizal associations were examined in the above mentioned plants. Some ECM fungi have dark mycelia that look like the mantle and Hartig net. Examples of these are *Kobresia filicina*, *K. myosuroides*, and *Polygonum viviparum*. Findings of this study revealed that *K. myosuroides* excelled in ECM root tip length, dry mass, and NH₄ concentration at -125 °C. Contrarily, *A. nitida* demonstrated the lower values, indicated its minimum tolerance. Notably, *T. repens* boasted the highest nitrogen concentration (18.7 ± 1.31 mg/g), while *P. sylvestris* led in phosphorus (3.2 ± 0.22 mg/g). The *B. pendula* showed the highest potassium concentration (9.4 ± 0.66 mg/g), emphasising species-specific nutrient uptake capabilities in extreme cold conditions. The PCA analysis revealed that the parameters, e.g., NH₄ in soil mix (NH₄), NO₃ in soil mix (NO₃), phosphorus in soil in species of *Polygonum viviparum*, *Kobresia filicina*, *K. myosuroides*, *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens* are most accurately represented in cases of +5 °C, -10 °C, and -20 °C temperatures. On the other hand, the parameters for ECM root tips (ECM) and Dry Mass (DM) are best described in -40 °C, -50 °C, and -125 °C temperatures. All parameters have a strong influence on the variability of the system indicated the efficiency of ECM. The heatmap supported the nutrients positively correlated with ECM colonization with the host plants.

*Correspondence:

Aisha Umar
ash.dr88@gmail.com

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Conclusion At lower temperatures, hyphae and spores in roots were reduced, while soluble phosphorus concentrations of leaves were increased in cold stress soils. Maximum foliar nutrient concentrations were found in *K. myosuroides* at the lowest temperature treatments due to efficient functioning and colonization of ECM.

Keywords Herbaceous plant, Fungi, Ectomycorrhiza, Hartig net, *Kobresia*, *Polygonum*, Altitude

Introduction

Shrub and tree roots are predominantly colonized by ectomycorrhizal (ECM and ECM-like) fungi, whereas they are rarely found in herbs. There are a few shrubs and herbaceous plants that make ECM colonization important for successional changes, e.g., *Arctostaphylos*, *Bistorta*, *Cistus*, *Helianthemum*, *Kobresia*, and *Salix herbacea* [1]. Ectomycorrhizal (ECM) relationships have changed their life styles in different types of plants and fungi [2]. Mycorrhizal herbs play an important role in maintenance and create ectomycorrhizal banks [3]. ECM roots are dominantly present in the upper organic layers of the soil (nearly 20 cm below the surface) [4, 5]. Ectomycorrhizal associations are found all over the world only in 8,000 groups of plants (about 3% of phanerogams) [1].

Plant species are busy to make the involvement with a huge range of Ascomycota and a few with Zygomycota fungal species [6, 7], while maximum association has been found with Basidiomycota [8]. Most of the ECM fungi can live on a wide range of host plants [9], but response is different for different plant species [10]. Vegetative mycelia spread out from the rhizosphere and act as a mycorrhizal inoculum for the nearby host plants [11] via connections with different plant species through a mycorrhizal network.

ECM are important for the natural growth, establishment, and restoration of woody plants, especially in areas damaged by human activities [12]. ECM associations play a prominent role in shaping the structure and movement of ECM plants and forest communities because they affect the soil environment and relationships between the plants [13]. ECM fungi are important for their plant partners for movement into a new area to start the successional processes and growth of plant communities [13]. ECM fungi can change themselves with the passage of time for individual roots, plant communities, and ecosystems as well [5]. Depending on the stage of succession and environmental history, ECM associations provide the different ecological functions and services [14]. ECM fungi are very essential for biogeochemical processes and plants to get nutrients [15]. Mountainous alpine and cold areas of Pakistan are hotspots for biodiversity.

A maximum number of valuable exclusive plants and fungal species are growing there. Adams et al. [3] suggested that ECM fungi frequently colonize herbaceous plants. In cold areas of Pakistan, the studies on the ectomycorrhizal relationship of herbaceous plants are limited

because mycorrhizal fungi with plants of cold areas are facing limited availability of essential nutrients. Cold areas are restricted in nutrients due to environmental conditions, so plant growth in such areas is limited due to the unavailability of nutrients [16]. For sake of their survival, these plants develop morpho-anatomical adaptations associated with fungi [17]. Fungal symbiotic association is an effective way to improve the supply of plants with nutrients [18]. The favourable reasons for the ECM relationship in plants of cold areas are abiotic stresses, lack of nutrition, and roots distribution pattern in the soil [19].

The uptake of nutrients through mycorrhizae in cold conditions may also be limited due to the inability of AM (arbuscular mycorrhizal) fungus to act well at low temperatures [20]. Cold climates provoke the nutrient demand in the plants, when the nutrient supply is limited in the soil [20, 21]. The ECM-plants symbiotic relationship is believed to be an effective association in alleviation of nutritional stress for both fungi and plants [22].

The organisms, which are adjusted in adverse conditions, tend to have a greater tolerance to the critical environmental factors than the species actually encounter in their habitats. The continuous exposure of organisms to low temperatures and freezing tolerance is usually fatal for the organism's survival [23]. In addition to ECM fungi, soil microbe communities are affected by the soil moisture and temperature, which leads to nutrient fluctuations. Frost affects the soil nutrient supply by physical breakage of organisms [24].

Here, we tested the hypothesis that ECM are more tolerant towards freezing temperatures and affect the availability of soil nutrients for plants. Moreover, in this study, different plant species were selected from such areas, where cold stress was more dominant, and examined the ectomycorrhizal relationship in the roots of plants along the nutrient's uptake.

Materials and methods

Sampling sites and plants collection

In this study, mycorrhizal associations of *Polygonum viviparum*, *Kobresia filicina*, *K. myosuroides*, *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens* were studied. *Kobresia* and *Polygonum* species from Deosai plains of Gilgit-Baltistan, while *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens* were collected from Hazara, Swat, Dir, and Bajaur,

respectively. In this study, selected plant root samples were removed from the depth of 0–15 cm of soil profile.

Morpho-anatomical mycorrhizal colonization

To determine whether the plants have ectomycorrhizal fungi, samples of root were taken from each plant species according to Baker [25]. Plant seedlings collected through the soil cores and ECM roots were washed with distilled water to remove soil particles. Heat treatment for one day of soil killed the endomycorrhizal fungi at 37 °C because ECM fungi were more competitive at lower temperatures [26]. Two subsamples of large root systems (3.0 cm and 6.5 cm depths) of at least 150 root tips from the selected plants per treatment per species were taken. The smallest root systems were needed to be fully observed and finally cut the roots into very small lengths. Clear samples were kept overnight in 10% KOH, 20 min in alkaline hydrogen peroxide, and 2 h in 1% hydrochloride before staining in a solution of glycerol, lactic acid, and methyl blue [27]. The ECM fungal contents of fine sieved roots were examined by dissection method [28] via MX4300H compound light microscope (Meiji Techo Co., Ltd., Japan). Data on anatomical features were recorded.

Turgid, healthy, ectomycorrhiza was fixed in 2% glutaraldehyde in 0.1 M HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (pH 6.5) for four hours, rinsed 5 to 7 times in buffer, dehydrated in ethanol, and embedded in gelatin capsules immediately after dehydration. Thin sections (1–1.2 mm) were cut into MT microtomes, stained for light microscopy with a solution of 1% (w/v) methylene blue, 1% (w/v) azure B, and 1% (w/v) sodium tetraborate in distilled water, and finally rinsed in distilled water. These were counterstained in 0.5% (w/v) basic fuchsin, air dried, mounted in immersion oil, and then observed under a photo microscope.

Soil preparation

The collected soil samples were sieved (6 mm) and homogenized by mixing the volume proportion of 1:2 (meadow soil:soil near the stands) to get soil that contained ECM inoculum. The mixed soil (4 cm thick layer) was placed in aluminium trays and each tray was placed under 6 temperature treatments (control+5 °C, -10 °C, -20 °C, -30 °C, -40 °C, -50 °C, and -125 °C). Temperature lowered first to +5 °C within 1 h and then decreased to 5 °C/h. Except that temperature was kept for 8 h at -3 °C before further lowering it to the target temperature (for 5 h) to ensure the soil was frozen. Subsequently, the temperature raised again at the rate of 5 °C/h.

At the end, the temperature was kept at +5 °C for 1 h before chamber opening. At -125 °C, the samples were first cooled to -50 °C with the above-described procedure in the same freezing chambers, similarly with other treatments. Afterward the temperature decreased to -125 °C

and then increased swiftly to -50 °C: 10 °C h⁻¹ in a “liquid-nitrogen cooled chamber (GCC30, Carbolite, Chelmsford, UK)”. The soil was then warmed from -50 to +5 °C (Arctest Chamber) at the rate of 5 °C h⁻¹. Treated soils and perlite (volume proportion of 30:70) were blended within each treatment.

Pot experiment

Plastic pots with 80 g of soil (water content 30% of dry matter) collected from the root zones of plant species in the filling volume of 185 mL were used. The pot height 80 mm, top diameter 60 mm and base diameter 48 mm. The five seeds of each plant species per treatment were grown in individual pots. Fifteen extra pots without seeds per treatment were left to check the treatments effect on the soluble nutrient concentrations in the soil. Thus, there were 5 (treatments) × 7 (plant species) × 10 (plant individuals) pots were placed in the growth room in randomized arrangement. Surface sterilized seeds of each plant were washed overnight under tap water before sowing. The next day, Tween 80 (one drop) was added and the seeds were shaken for 5 min. Next, seeds were soaked in 30% H₂O₂ for 20 min and were rinsed 5 times in distilled water and 5 seeds were placed in each pot. Covered the seeds in a thin soil layer and germinated in a growth chamber under fluorescent tubes (VHO 215 W, Sylvania Cool White, Sylvania, USA); relative humidity (90%), 22 °C, 20 h day, 4 h night.” After germination, the growth conditions were 20 h day at 22 °C, 60% relative humidity, and 4 h night at 17 °C in 80% relative humidity. The cooling/warming rate was 5 °C/h. Day/night irradiance was ca. 350 μmol m⁻²s⁻¹ PAR from incandescent lamps and tubes. Light intensity was changed stepwise in the beginning and end of a day for 2 h.

At the start of 6.5 weeks, the seedlings were thinned to one (per pot), and 4.5 weeks after the sowing, fertilizer N-P-K was applied with all other nutrients. By this, ‘N’ dose per week was 1 mg in 10 mL water. Watered all pots till those reached the 60% water holding capacity, and plants were harvested 11 weeks after the sowing.

Soil properties

Without frost treatments of the soil mixture, dry matter content (105 °C) was estimated. For water holding capacity (of soil mixture): weighed out the five wet filter papers and placed in funnels (equipped with rubber tubes and pinch clamps), where 5 g soil mixture in 100 mL water was presented. For 2 h, soils were incubated, and after that, slightly clamps opened until the water flow ceased (ca. 30 min). Soil saturated with water was weighed out. The soil samples were dried at 105 °C and weighed out again. The water holding capacity of the soil was calculated (per dry matter). Dry (40 °C) biomass of seedling parts was also estimated.

Plant nutrients

Total nitrogen concentration of initial soil, soil mix, and plants was determined by Kjeldahl method [30]. The other nutrients were determined by “ICP-OES (Iris Intrepid II XSP, Thermo Elemental, Franklin, MA, USA)” after MARS5 microwave wet digestion in HNO_3 and H_2O_2 in Teflon containers. For soil nutrient analysis, 75 pots were watered like other pots during the growing period without fertilization. At the same time, soil was sampled with plant harvest, dried (40 °C), and dried matter content (105 °C) was determined. Soil (10 g) was mixed in 1 M KCl (100 mL) for 1 h. Filtered the extracts, and $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations were calculated by the flow injection analyzer “(FIAstar 5012, Tecator, Sweden)”. Soil of the 75 pots was analyzed for other nutrients (ICP-OES after ammonium acetate extraction) at pH 4.65.

Statistical analysis

A one-way ANOVA was used to compare numeric variables across different groups. Post-hoc comparisons using the Least Significant Difference (LSD) were conducted to determine the pairwise differences. If the difference between two means was greater than the LSD value, that difference was considered statistically significant at a level of 0.05. The significance level (α) was set at 0.05, indicated a p -value less than 0.05 considered statistically significant. SPSS 28 IBM software for Windows was used for the statistical analysis.

Principal component analysis was used to determine the relationships between the studied cases and parameters. Statistical software (version 12.0, StatSoft Inc., Tulsa, OK, USA) was used for statistical analysis. Principal components analysis (PCA) was performed at the significance level, and the matrix of data used for the PCA statistical analysis of the temperature test resulted in 7 rows and 36 columns for ECM root tips: Dry Mass (DM), NH_4 in soil mix (NH_4), NO_3 in soil mix (NO_3), and Phosphorus in soil (P). The matrix for the statistical analysis of chemical elements had 7 rows and 15 columns for concentrations and for content of nutrients in plant leaves. The optimal number of main components obtained in both cases was determined based on the Cattel criterion. The input matrix has been automatically rescaled.

Results

Cold stressed plants and ECM colonization

The species of plants are commonly found in cold alpine and stress-inducing habitats. Fungus made anatomical structures with *K. filicina* (Fig. 1A), *K. myosuroides* (Fig. 1B), and *P. viviparum* (Fig. 1C) herbaceous plant species of stress-making habitats. In this study, *K. filicina*, *K. myosuroides*, and *P. viviparum* showed the ECM associations with ascomycete fungus (*Cenococcum*

geophilum) rather than endomycorrhizal type (Fig. 1D). Endomycorrhizal type was absent in these targeted species. Spherical cleistothecia of *C. geophilum* was covered by the dark pigmented hyphae (groups of isodiametric cells) encircled by elongated cells in a stellate pattern. A few parts of cleistothecia were covered by emanating hyphae with rounded bases, and a few rounded cells encapsulated by hyphae (gelatinous in wet but hard when dry) in a dense network form (Fig. 1D1). The stiff, simple, septate, and without clamp connections hyphae of the ECM fungus were found in these plant species (Fig. 1D2).

Cells of mantle formed by fungal species were extended by bristle-like hyphae (two-layered and thick-walled). The extended bristle-like hyphae were septate, while simple pores were seen in the hyphae of the mantle and Hartig net. Mantle cells were compactly arranged without intercellular spaces. Fungal cells were penetrated longitudinally in the outer cortical cells of the root and then into the inner cells of the root to form the Hartig net. The hyphal walls of Hartig net were thin, without intercellular spaces, uniform, and uniformly contacted the adjacent walls of cortical cells (Fig. 1D1, D2).

Specialized dauciform roots were seen in *K. filicina* (Fig. 2A). These roots are colonized by ECM fungus, facilitating the supply of nutrients from the nutrients deficient soil. The shape of the dauciform roots resembled the carrots, and its surface was free from root hairs (Fig. 2A1). In this study, dauciform roots were beige in colour. The originating pattern of these roots was lateral and appeared like a peduncle or non-peduncle from the main plant root. Hartig net was not seen in this root type (Fig. 2A1). Ectomycorrhiza of *Cenococcum geophilum* in *K. myosuroides* (Fig. 2B) formed the epidermal Hartig net and fungal mantle (Fig. 2B1). Hyphae of Hartig net were radially elongated in root epidermal cells, and the mantle formed by many layers of compactly organized hyphae.

Polygonum viviparum was strongly influenced by the ECM fungal colonization (Fig. 2C). Simple, club-shaped, cylindrical, and bright-coloured ectomycorrhizal fungal hyphae were observed in *P. viviparum*. The *P. viviparum* roots were a network of several mycorrhizal lateral roots (both short and long) that emerged from the plant's tiny propagules (bulbils generated in cold conditions). Due to different *Cenococcum* species, some ECM have light-coloured mantles, while others have dark-coloured mantles. The mantle (Fig. 2C1) covered the root apex and initiated a distinct epidermal Hartig net near the apical meristem. A variety of ECM has polysaccharide-rich mantles. The root cap was reduced by the plants' interaction with fungal species. Hartig net cells were penetrated in the single elongated layer of root epidermal cells (Fig. 2C2).

Cenococcum fungus happily associated the *K. myosuroides*, *K. filicina*, and *P. viviparum*. Mantle texture and

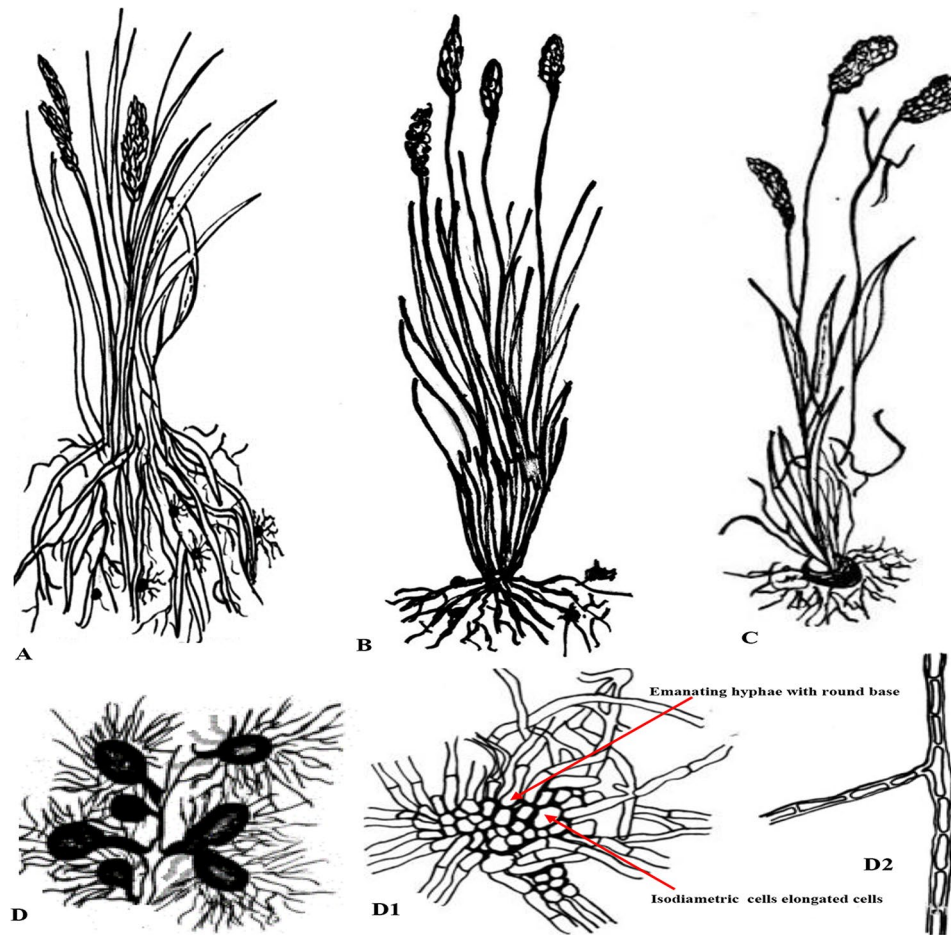


Fig. 1 Plant root system with ECM fungal species **A.** *Kobresia filicina*, **B.** *Kobresia myosuroides*, **C.** *Polygonum viviparum*, and **D.** Mycelium of *Cenococcum geophilum* (**D1.** Spherical cleistothecia **D2.** Thick-walled mycelium, two-layered)

colour of *K. myosuroides* and *P. viviparum* depended on ECM morphotypes. The orchestration of ECM fungal anatomical structures depends upon the hyphal division, behaviour, rearrangements, and cell shape.

Effects of temperature on ECM roots

The length of ECM root tips was measured under different temperature conditions (-10 °C, -20 °C, -30 °C, -40 °C, -50 °C, and -125 °C) and compared to a control temperature of 5 °C. Statistical analysis, including mean and standard deviation calculations were performed. The differences in mean ECM root tip length between the temperature conditions were evaluated using the LSD (Least Significant Difference) test. The length of ECM root tips varied among the different temperature conditions and plant species. At the control temperature of 5 °C, *Polygonum viviparum* had a mean ECM root tip length of 40.0 ± 2.8 mm. The length increased to 52.0 ± 3.6 mm at -10 °C, 54.0 ± 3.8 mm at -20 °C, 57.0 ± 4.0 mm at -30 °C, 60.0 ± 4.2 mm at -40 °C, 62.0 ± 4.3 mm at -50 °C, and 64.0 ± 4.5 mm at -125 °C. If the difference between two means was greater than the LSD value, the difference

was considered statistically significant at a level of 0.05 (Table 1).

Similarly, other plant species, such as *Kobresia filicina*, *K. myosuroides*, *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens*, showed variations in mean ECM root tip length across different temperature conditions. The mean ECM root tip lengths generally increased with decreasing temperature.

Effects of temperature on dry mass

The dry mass measurements were taken at different temperature conditions (-10 °C, -20 °C, -30 °C, -40 °C, -50 °C, and -125 °C) and compared to a control temperature of 5 °C. The dry mass of the plant species exhibited variations under different temperature conditions. For *Polygonum viviparum*, the lowest mean dry mass was observed at the control temperature (5 °C) with a value of 2 ± 0.14 g. It increased to 2.2 ± 0.15 g at -10 °C, 2.4 ± 0.17 g at -20 °C, 2.6 ± 0.18 g at -30 °C, 2.8 ± 0.2 g at -40 °C, 3 ± 0.21 g at -50 °C, and 3.2 ± 0.22 g at -125 °C.

The standard deviations (SD) for these measurements ranged from 0.13 to 0.22 g. The LSD analysis indicated

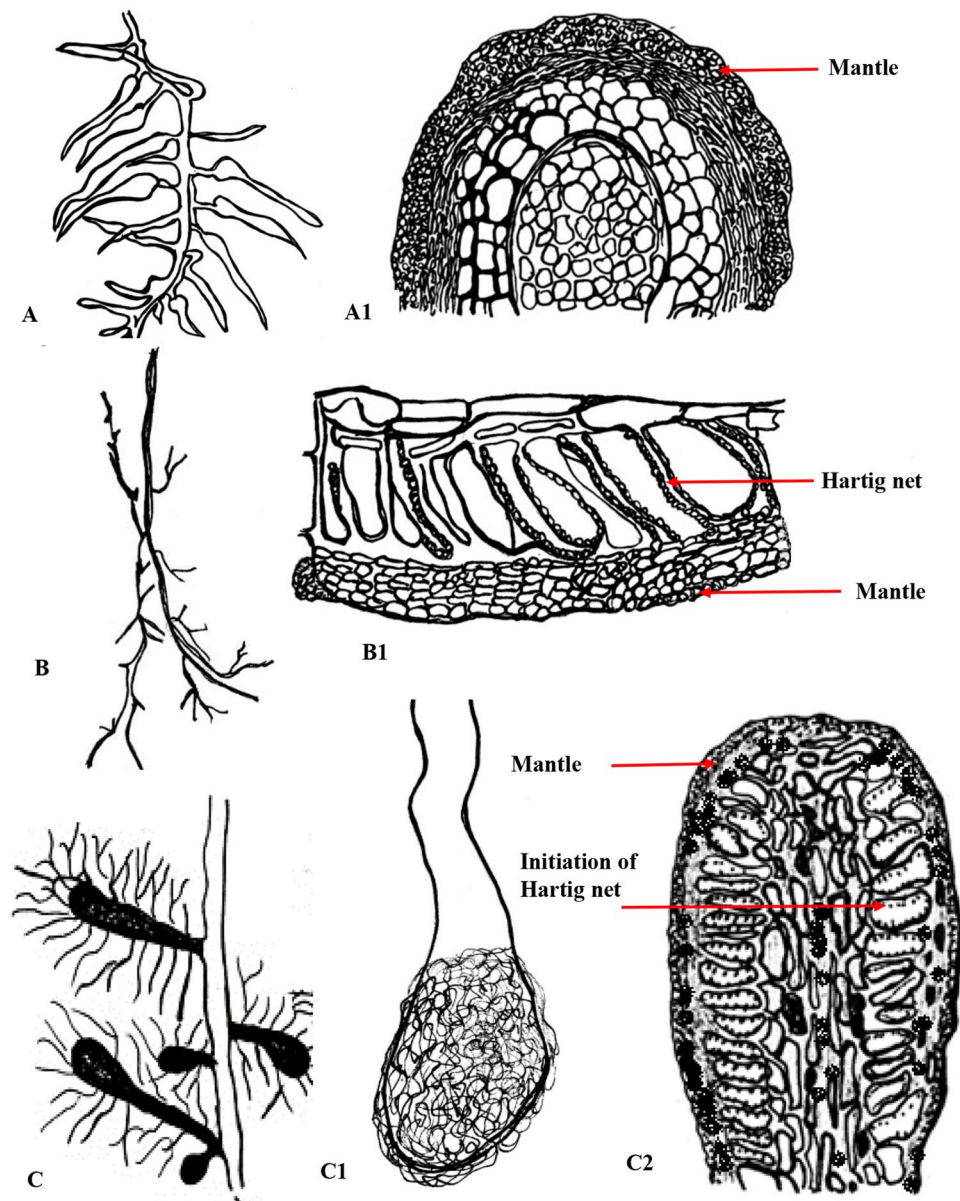


Fig. 2 (A) Dauciform roots of *K. filicina*. **A1**. Transverse section of a dauciform root with mantle. (B) Mycorrhizal roots of *K. myosuroides*. **B1**. Arrow indicated compact mantle and radially enlarged epidermal cells and well-developed Hartig net of *K. myosuroides*. (C) Mycorrhizal roots of *P. viviparum*. **C1**. Dark mycorrhizal fungal mantle of *P. viviparum*. **C2**. Two-layered ectomycorrhiza mantle (arrowed), initiation of Hartig net (arrowed), and radially elongated epidermal cells (red arrowed) of *P. viviparum*

that the differences in mean dry mass between the control temperature and -10 °C, -20 °C, -30 °C, -40 °C, -50 °C, and -125 °C were statistically significant, with LSD values of 0.61, 0.25, 0.13, 0.02, 0.18, and 0.15, respectively ($p < 0.05$).

Kobresia filicina also showed variations in mean dry mass across different temperature conditions. The mean dry mass was lowest at the control temperature (5 °C) with a value of 1.1 ± 0.08 g. It increased to 1.5 ± 0.11 g at -10 °C, 2.2 ± 0.15 g at -20 °C, 3 ± 0.21 g at -30 °C,

3.5 ± 0.25 g at -40 °C, 3.8 ± 0.27 g at -50 °C, and 4.1 ± 0.29 g at -125 °C.

The standard deviations for these measurements ranged from 0.08 to 0.29 g. The LSD test revealed that the differences in mean dry mass between the control temperature and -10 °C, -20 °C, -30 °C, -40 °C, -50 °C, and -125 °C were statistically significant, with LSD values of 0.61, 0.25, 0.13, 0.02, 0.18, and 0.15, respectively ($p < 0.05$) (Table 2). Similar trends were observed for the other plant species, *Kobresia myosuroides*, *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens*.

Table 1 Effects of temperature on ECM Root tip length in different plant species

Temp	(ECM root tips)						
	<i>Polygonum viviparum</i>	<i>Kobresia filicina</i>	<i>Kobresia myosuroides</i>	<i>Alnus nitida</i>	<i>Betula pendula</i>	<i>Pinus sylvestris</i>	<i>Trifolium repens</i>
5 °C	40.0±2.8	65.0±5.2	60.0±4.2	29.0±2.0	52.0±3.6	40.0±2.8	30±2.4
-10 °C	52.0±3.6	70.0±4.9	62.0±4.3	32.0±2.2	53.0±4.2	43.0±3.0	32±2.2
-20 °C	54.0±3.8	72.0±6.5	65.0±4.6	34.0±2.7	54.0±3.8	45.0±3.2	34±1.7
-30 °C	57.0±4.0	75.0±6.0	67.0±4.7	35.0±2.8	54.0±3.2	48.0±3.4	36±2.9
-40 °C	60.0±4.2	78.0±4.7	69.0±4.8	38.0±2.7	57.0±4.0	49.0±3.4	38±2.7
-50 °C	62.0±4.3	80.0±6.4	70.0±4.9	40.0±2.8	59.0±4.7	50.0±3.5	39±2.7
-125 °C	64.0±4.5	82.0±5.7	75.0±5.3	42.0±2.9	60.0±4.2	52.0±3.6	40±3.2
LSD	3.89	4.08	3.16	2.21	2.61	2.2	2.54

Results are (means±S.D.) (n=3), *Significance difference of ECM root tips for each plant species at different cold temperatures *p*-value<0.05. Control temperature (5 °C)

Table 2 Effects of temperature on dry mass of the plant species in soil mix

Temp	Dry mass						
	<i>Polygonum viviparum</i>	<i>Kobresia filicina</i>	<i>Kobresia myosuroides</i>	<i>Alnus nitida</i>	<i>Betula pendula</i>	<i>Pinus sylvestris</i>	<i>Trifolium repens</i>
5 °C	2±0.14	1.1±0.08	1.3±0.09	0.5±0.04	0.2±0.01	0.4±0.03	1.5±0.11
-10 °C	2.2±0.15	1.5±0.11	1.4±0.1	0.7±0.05	0.3±0.02	0.5±0.04	1.4±0.1
-20 °C	2.4±0.17	2.2±0.15	1.6±0.11	0.9±0.06	0.5±0.04	0.6±0.04	1.2±0.08
-30 °C	2.6±0.18	3±0.21	1.8±0.13	0.8±0.06	0.7±0.05	0.7±0.05	1.1±0.08
-40 °C	2.8±0.2	3.5±0.25	2±0.14	1±0.07	0.8±0.06	0.7±0.05	0.9±0.06
-50 °C	3±0.21	3.8±0.27	2.8±0.2	1.5±0.11	0.9±0.06	0.8±0.06	0.5±0.04
-125 °C	3.2±0.22	4.1±0.29	2.9±0.2	2±0.14	1±0.07	0.9±0.06	0.4±0.03
LSD	0.61	0.25	0.13	0.02	0.18	0.11	0.15

Results are (means±S.D.) (n=3), *Significance difference of Dry Mass for each plant species at different cold temperatures *p*-value<0.05. Control temperature (5 °C)

Table 3 Effects of temperature on NH₄ concentrations in soil mix

Temp.	NH ₄ in soil mix						
	<i>Polygonum viviparum</i>	<i>Kobresia filicina</i>	<i>Kobresia myosuroides</i>	<i>Alnus nitida</i>	<i>Betula pendula</i>	<i>Pinus sylvestris</i>	<i>Trifolium repens</i>
5 °C	70±4.9	60±4.2	80±5.6	20±1.4	50±3.5	45±3.2	40±2.8
-10 °C	65±4.6	58±4.1	75±5.3	18±1.3	45±3.2	43±3	38±2.7
-20 °C	56±3.9	56±3.9	64±4.5	15.6±1.1	42±2.9	40±2.8	37.8±2.6
-30 °C	52±3.6	55±3.9	60±4.2	15±1.1	40±2.8	37±2.6	37±2.6
-40 °C	50±3.5	40±2.8	58±4.1	14±1	25±1.8	30±2.1	45±3.2
-50 °C	42±2.9	32±2.2	55±3.9	12±0.8	22±1.5	24±1.7	32±2.2
-125 °C	40±2.8	30±2.1	50±3.5	15±1.1	20±1.4	20±1.4	30±2.1
LSD	2.7	3.12	3.48	1.19	2.3	3.18	2.7

Results are (means±S.D.) (n=3), *Significance difference of NH₄ in soil mix for each plant species at different cold temperatures *p*-value<0.05. Control temperature (5 °C)

The mean dry mass generally increased with decreasing temperature.

Effects of temperature on ammonium (NH₄) concentrations in soil mix

This study aimed to examine the effects of different temperature conditions on ammonium concentrations in a soil mix. The mean ammonium concentrations were determined for various plant species (*Polygonum viviparum*, *Kobresia filicina*, *K. myosuroides*, *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens*) under different temperature conditions (-10 °C, -20 °C, -30 °C, -40 °C, -50 °C, and -125 °C). The mean ammonium concentrations varied among the

temperature conditions for each plant species. For *Polygonum viviparum*, the highest mean ammonium concentration was observed at the control temperature (5 °C) with a value of 70±4.9 mg/kg. It decreased to 65±4.6 mg/kg at -10 °C, 56±3.9 mg/kg at -20 °C, 52±3.6 mg/kg at -30 °C, 50±3.5 mg/kg at -40 °C, 42±2.9 mg/kg at -50 °C, and 40±2.8 mg/kg at -125 °C.

The standard deviations (SD) for these measurements ranged from 2.1 to 4.9. The LSD analysis revealed that the differences in mean ammonium concentrations between the control temperature and -10 °C, -20 °C, -30 °C, -40 °C, -50 °C, and -125 °C were statistically significant, with LSD values of 2.7, 3.12, 3.48, 1.19, 2.3, and 2.7, respectively (*p*<0.05) (Table 3).

Kobresia filicina also exhibited variations in mean ammonium concentrations across different temperature conditions. The mean ammonium concentration was highest at the control temperature (5 °C) with a value of 60±4.2 mg/kg. It decreased to 58±4.1 mg/kg at -10 °C, 56±3.9 mg/kg at -20 °C, 55±3.9 mg/kg at -30 °C, 40±2.8 mg/kg at -40 °C, 32±2.2 mg/kg at -50 °C, and 30±2.1 mg/kg at -125 °C.

The standard deviations for these measurements ranged from 2.1 to 4.2. The LSD test indicated that the differences in mean ammonium concentrations between the control temperatures, -10 °C, -20 °C, -30 °C, -40 °C, -50 °C, and -125 °C were statistically significant, with LSD values of 2.7, 3.12, 3.48, 1.19, 2.3, and 2.7, respectively ($p<0.05$) (Table 3). Similar patterns were observed for the other plant species, *Kobresia myosuroides*, *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens*. The mean ammonium concentrations decreased with decreasing temperature.

Effects of temperature on nitrate (NO₃) concentrations in soil mix

The effects of different temperature conditions on nitrate concentrations in a soil mix were determined in this work. The mean nitrate concentrations were measured for various plant species under different temperature conditions (-10 °C, -20 °C, -30 °C, -40 °C, -50 °C, and -125 °C). The mean nitrate concentrations varied among the temperature conditions for each plant species. For *Polygonum viviparum*, the mean nitrate concentration was highest at the control temperature (5 °C) with a value of 50 mg/kg, and it decreased to 45 mg/kg at -10 °C, 42 mg/kg at -20 °C, 40 mg/kg at -30 °C, 25 mg/kg at -40 °C, 22 mg/kg at -50 °C, and 20 mg/kg at -125 °C. The standard deviations (SD) for these measurements ranged from 1.4 to 3.5. The LSD analysis revealed that the differences in mean nitrate concentrations between the control temperature and -10 °C, -20 °C, -30 °C, -40 °C, -50 °C, and -125 °C were statistically significant, with LSD values of 2.31, 2.51, 2.54, 2.84, 1.58, and 1.7, respectively. *Kobresia*

filicina also exhibited variations in mean nitrate concentrations across different temperature conditions. The mean nitrate concentration was highest at the control temperature (5 °C) with a value of 40 mg/kg. It decreased to 37 mg/kg at -10 °C, 36 mg/kg at -20 °C, 33.5 mg/kg at -30 °C, 33 mg/kg at -40 °C, 32 mg/kg at -50 °C, and 30 mg/kg at -125 °C. The standard deviations for these measurements ranged from 2 to 3.2. The LSD test indicated that the differences in mean nitrate concentrations between the control temperature and -10 °C, -20 °C, -30 °C, -40 °C, -50 °C, and -125 °C were statistically significant, with LSD values of 2.31, 2.51, 2.54, 2.84, 1.58, and 1.7, respectively ($p<0.05$) (Table 4).

Effects of temperature on Phosphorus concentrations in soil

The mean phosphorus concentrations were measured for plant species (*Polygonum viviparum*, *Kobresia filicina*, *K. myosuroides*, *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens*) under different temperature conditions (-10 °C, -20 °C, -30 °C, -40 °C, -50 °C, and -125 °C). Statistical analysis, including one-way analysis of variance (ANOVA) and post-hoc tests was performed to assess the significance of the differences in mean phosphorus concentrations among the temperature conditions. The mean phosphorus concentrations varied among the temperature conditions for each plant species. For *Polygonum viviparum*, the mean phosphorus concentration was 14 mg/kg at control temperature (5 °C), but it decreased to 12 mg/kg at -10 °C and further declined to 8.5 mg/kg at -40 °C. The LSD analysis revealed that the difference between the mean phosphorus concentrations at control temperature and -10 °C was statistically significant (LSD=1.73, $p<0.05$). Similarly, a significant difference was observed between the mean phosphorus concentration at control temperature and -40 °C (LSD=1.73, $p<0.05$). *Kobresia filicina* exhibited a different pattern, with the mean phosphorus concentration being 14 mg/kg at control temperature, 11 mg/kg at -10 °C, and 6 mg/kg at -50 °C. The LSD test indicated that

Table 4 Effects of temperature on nitrate concentrations in soil mix

Temp.	NO ₃ in soil mix						
	<i>Polygonum viviparum</i>	<i>Kobresia filicina</i>	<i>Kobresia myosuroides</i>	<i>Alnus nitida</i>	<i>Betula pendula</i>	<i>Pinus sylvestris</i>	<i>Trifolium repens</i>
5 °C	50±3.5	40±3.2	45±3.2	40±2.8	20±1.4	30±2.1	20±1.6
-10 °C	45±3.2	37±2.6	43±3	38±2.7	18±1.4	18±1.3	19±1.3
-20 °C	42±2.9	36±3.2	40±2.8	37.8±3	15.6±1.1	15.6±1.2	18.9±1.9
-30 °C	40±2.8	33.5±2.7	37±2.6	37±3	15±2.3	15±1.3	18±1.4
-40 °C	25±1.8	33±2	30±2.1	45±3.2	14±1.6	14±1.4	16±1.6
-50 °C	22±1.5	32±2.6	24±1.7	32±2.2	12±1.9	12±1.8	15.5±1.8
-125 °C	20±1.4	30±2.1	20±1.4	30±2.1	15±1.3	20±1.4	15±1.2
LSD	2.31	2.51	2.54	2.84	1.58	1.7	1.54

Results are (means±S.D.) (n=3), *Significance difference of NO₃ in soil mix for each plant species at different cold temperatures p -value<0.05. Control temperature (5 °C)

Table 5 Effects of temperature on phosphorus concentrations in soil

Temp.	Phosphorus in soil						
	<i>Polygonum viviparum</i>	<i>Kobresia filicina</i>	<i>Kobresia myosuroides</i>	<i>Alnus nitida</i>	<i>Betula pendula</i>	<i>Pinus sylvestris</i>	<i>Trifolium repens</i>
5 °C	14±0.98	14±0.98	16±1.12	10±0.7	15±1.05	12±0.84	10±0.7
-10 °C	12±0.84	11±0.77	15±1.05	8.9±0.62	10.5±0.74	10±0.7	9±0.63
-20 °C	11±0.77	10±0.7	14.5±1.02	7.5±0.53	9±0.63	9.6±0.67	8.6±0.6
-30 °C	9±0.63	7.8±0.55	14±0.98	8±0.56	8±0.56	9±0.63	7.5±0.53
-40 °C	8.5±0.6	6±0.42	12±0.84	6.5±0.46	7±0.49	8.4±0.59	7±0.49
-50 °C	7±0.49	5±0.35	9±0.63	9.9±0.69	6.5±0.46	8±0.56	6.8±0.48
-125 °C	5±0.35	3±0.21	8±0.56	7±0.49	6±0.42	6±0.42	5±0.35
LSD	1.73	1.23	2.17	1.35	1.39	1.71	1.02

Results are (means±S.D.) (n=3), *Significance difference of Phosphorus in Soil for each plant species at different cold temperatures p -value<0.05. Control temperature (5 °C)

Table 6 Nutrients concentrations in plant leaves of different species at -125 °C

	Mean conc. (mg g ⁻¹) of nutrients in plants leaves at -125 °C							LSD
	<i>Polygonum viviparum</i>	<i>Kobresia filicina</i>	<i>Kobresia myosuroides</i>	<i>Alnus nitida</i>	<i>Betula pendula</i>	<i>Pinus sylvestris</i>	<i>Trifolium repens</i>	
N	20±1.40	16±1.12	15.5±1.09	17.7±1.24	11±0.77	14.6±1.02	18.7±1.31	1.14
P	2.5±0.18	1.5±0.11	2.1±0.15	1.4±0.10	2.4±0.17	3.2±0.22	2.2±0.15	1.02
K	8.7±0.61	7.7±0.54	6.5±0.46	8.6±0.60	9.4±0.66	7.4±0.52	8.5±0.60	2.57
Ca	5.4±0.38	8.5±0.60	3.5±0.25	4.6±0.32	4.5±0.32	4.8±0.34	4.6±0.32	1.36
Cu	4.7±0.33	4.8±0.34	7.3±0.51	6.5±0.46	3.8±0.27	8.5±0.60	7.6±0.53	1.72
Mn	300±5.00	97.5±6.83	205±4.60	150±6.50	400±7.5	200±4.90	160±4.20	14.27
Zn	200±4.30	38±2.66	150±5.20	70±4.90	180±6.2	170±6.30	76±5.32	9.57

Results are (means±S.D.) (n=3), *Significance difference of nutrients concentrations in each plant leaves species at forest temperature (-125 °C), p -value<0.05

the difference between the mean phosphorus concentrations at control temperature and -10 °C was statistically significant (LSD=1.23, p <0.05). Likewise, a significant difference was found between the mean phosphorus concentration at control temperature and -50 °C (LSD=1.23, p <0.05) (Table 5).

The other plant species (*Kobresia myosuroides*, *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens*) also showed variations in mean phosphorus concentrations across different temperature conditions. However, the differences were not statistically significant based on the LSD values calculated at a significance level of 0.05 (LSD values ranging from 1.02 to 2.17).

Nutrients concentrations in plant leaves

This study aimed to investigate the nutrient concentrations in the leaves of various plant species at an extreme cold temperature (-125 °C). The mean concentrations and standard deviations (SD) of nutrients were measured for seven plant species, including *Polygonum viviparum*, *Kobresia filicina*, *K. myosuroides*, *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens*. The statistical significance of the observed differences was evaluated using a significance level of 0.05. The results revealed the variations in nutrient concentrations among the different plant species at -125 °C. Each nutrient showed distinct patterns across the species.

Nitrogen (N) in *P. viviparum* exhibited the highest mean

concentration (1.40 mg g⁻¹), while *B. pendula* showed the lowest (11 mg g⁻¹) concentration. If the difference in mean 'N' concentrations between two plant species greater than 1.14, the difference was considered statistically significant at a significance level of 0.05 (Table 6).

Phosphorus (P) concentrations ranged from 1.4 to 3.2 mg g⁻¹, with *K. filicina* having the lowest mean concentration (1.5 mg g⁻¹) and *P. sylvestris* having the highest (3.2 mg g⁻¹) values. If the difference in mean 'P' concentrations between two plant species was greater than 1.02, this difference was considered statistically significant at a significance level of 0.05.

Potassium (K) concentrations varied from 6.5 to 9.4 mg g⁻¹, with *K. myosuroides* having the lowest mean concentration (6.5 mg g⁻¹) and *B. pendula* having the highest (9.4 mg g⁻¹). If the difference in mean 'K' concentrations between two plant species was greater than 2.57, the difference was considered statistically significant at a significance level of 0.05.

Calcium (Ca) concentrations ranged from 3.5 to 8.5 mg g⁻¹, with *K. myosuroides* exhibited the lowest mean concentration (3.5 mg g⁻¹) and *K. filicina* having the highest (8.5 mg g⁻¹). If the difference in mean 'Ca' concentrations between two plant species greater than 1.36, the difference was considered statistically significant at a significance level of 0.05.

Copper (Cu) concentrations varied from 3.8 to 8.5 mg g⁻¹, with *T. repens* exhibited the lowest mean concentration (3.8 mg g⁻¹), while *P. sylvestris* has highest (8.5 mg g⁻¹) concentration. If the difference in mean 'Cu' concentrations between two plant species greater than 1.72, the difference was considered statistically significant at a level of 0.05.

Manganese (Mn) concentrations ranged from 97.5 to 400 mg g⁻¹, *K. filicina* presented the lowest mean concentration (97.5 mg g⁻¹) and *P. viviparum* having the highest (400 mg g⁻¹). If the difference in mean 'Mn' concentrations between two plant species was greater than 14.72, the difference was considered statistically significant at a significance level of 0.05 (Table 6).

Principal component analysis (PCA) for temperature

Performing principal component analysis (PCA) allowed us to obtain six new variables that explained 100% of the variability of the system. The PCA analysis showed that the first two main components PC1 and PC2 explained 93.13% of the variability of the system. All parameters except *Pinus sylvestris* for NO₃ in soil mix have a strong influence on the variability of the system because they fall between the two red circles (Fig. 3A). This parameter also affects the variability of the system, but it was not as strong as other parameters. The parameters for NH₄ in soil mix (NH₄), NO₃ in soil mix (NO₃), Phosphorus in soil (P): *Polygonum viviparum*, *Kobresia filicina*, *K. myosuroides*, *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens* were strongly and positively correlated. *Trifolium repens*, except: NO₃ *Alnus nitida* and NO₃ *Pinus sylvestris* for NO₃ in soil mix (NO₃), NH₄ *Trifolium repens* for NH₄ in soil mix, and 'P' *Alnus nitida* for Phosphorus in soil.

There was also a positive and strong correlation between the parameters for ECM root tips (ECM), Dry Mass (DM): *P. viviparum*, *K. filicina*, *K. myosuroides*, (A) *nitida*, (B) *pendula*, *P. sylvestris*, and *T. repens*. A strong and negative correlation was observed between the parameters for ECM root tips (ECM), Dry Mass (DM), and the parameters for NH₄ in soil mix (NH₄), NO₃ in soil mix (NO₃), Phosphorus in soil (P) except NO₃ *Alnus nitida*, NO₃ *Pinus sylvestris*, NH₄ *Trifolium repens*, 'P' *Alnus nitida*.

The PCA analysis also showed that the first principal component (PC1) described 86.16% of the differences between the parameters for ECM root tips (ECM), Dry Mass (DM) and NH₄ in soil mix (NH₄), NO₃ in soil mix (NO₃), Phosphorus in soil (P) (Fig. 3B). Positive values of the main component PC1 described the results for ECM root tips (ECM), Dry Mass (DM), and negative values of the main component PC1 explained the

results parameters for NH₄ in soil mix (NH₄), NO₃ in soil mix (NO₃), and Phosphorus in soil (P). The second principal component (PC2) in 6.97% described the differences between NO₃ *Alnus nitida*, NH₄ *Trifolium repens*, NO₃ *Pinus sylvestris*, and 'P' *Alnus nitida*. Positive values of the main component PC2 described the results for NO₃ *Alnus nitida* and NH₄ *Trifolium repens*, and negative values of the main component PC2 described the results for 'NO₃' *Pinus sylvestris* and 'P' *Alnus nitida*.

The PCA analysis (Fig. 3A, B) indicated that parameters for NH₄ in soil mix, NO₃ in soil mix, phosphorus in soil, *Polygonum viviparum*, *Kobresia filicina*, *K. myosuroides*, *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens* were best described by conditions at 5 °C, -10 °C, and -20 °C, while parameters for ECM root tips and dry mass were best described by -40 °C, -50 °C, and -125 °C.

Principal component analysis (PCA) for chemical elements

Performing principal component analysis (PCA) allowed us to obtain six new variables that explained 100% system variability. The PCA analysis showed that the first two main components PC1 and PC2 explained 99.59% of the variability of the system. All parameters have a strong influence on the variability of the system because these all presented in the red circle (Fig. 4A). The parameters for the concentration in mg g⁻¹ of nutrients in plant leaves at -125 °C were strongly and positively correlated: *Polygonum viviparum*, *Kobresia filicina*, *K. myosuroides*, *Alnus nitida*, *Betula pendula*, *Pinus sylvestris*, and *Trifolium repens*. There was also a positive and strong correlation for the content in 'mg' of nutrients in plant leaves at -125 °C between the parameters: *P. viviparum*, *K. filicina*, *K. myosuroides*, (A) *nitida*, (B) *pendula*, *P. sylvestris*, and *T. repens*. No correlation was observed between these parameters for the concentration in mg g⁻¹ of nutrients in plant leaves at -125 °C, and for the content in 'mg' of the nutrients in plant leaves at -125 °C. A more homogeneous group was formed by the parameters for the content than for the concentration of nutrients in plant leaves at -125 °C.

The PCA analysis exhibited that the first principal component (PC1) described the differences between 'Ca, Cu, K, N, P, Mn, and Zn' in 64.17% (Fig. 4B). Positive values of the PC1 principal component explained the results for Ca, Cu, K, N, and P, and negative values of the PC1 described the results for Mn and Zn. The second principal component (PC2) exhibited the differences between Mn and Zn in 35.43%. Positive values of the main component PC2 described the results for 'Mn', and negative values of the main component PC2 represented the 'Zn'.

The PCA analysis (Fig. 4A, B) also explained that the parameters of *P. viviparum*, *K. filicina*, *K. myosuroides*, (A) *nitida*, (B) *pendula*, *P. sylvestris*, and *T. repens*

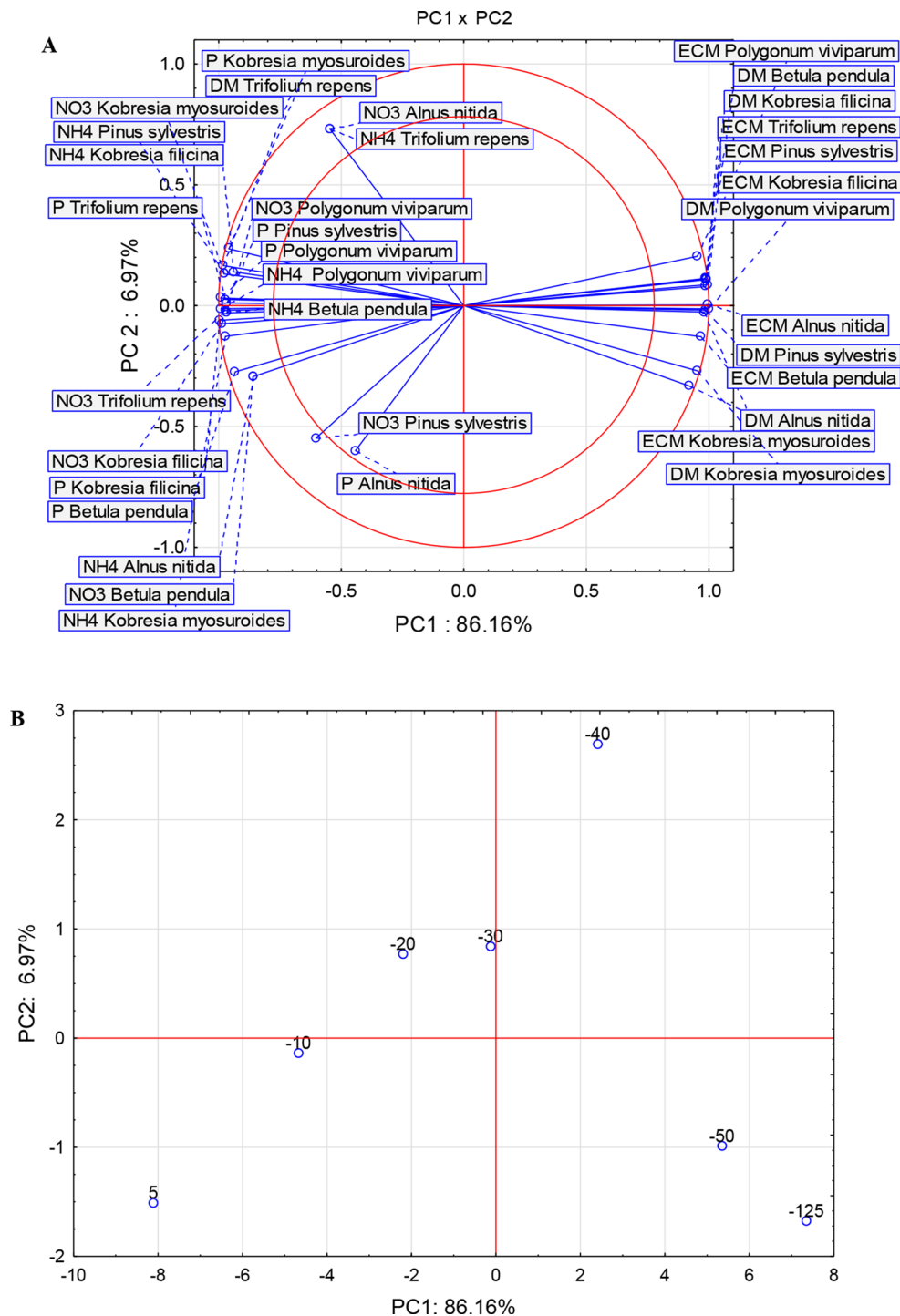


Fig. 3 (A) Projection of variables: parameters on the PC1 and PC2 loadings plot, (B) Projection of sample type on the PC1 and PC2 scores plot

for concentration in mg g^{-1} of nutrients in plant leaves at -125°C and best described the 'Mn' and the parameters for the content in mg of nutrients in plant leaves at -125°C showed excellence for Zn. No significant differences were observed in the concentration and content of parameters in the case of Ca, Cu, K, N, and P.

Correlation

Combinations of pair of variables were mentioned in Table 7 (Table S1) of the heat map. The table's row and column headers comprised the name of the variables and numerical values of the calculated correlation coefficients (inside the tables). The value of the correlation coefficient was closed in the range of -1, 1. The larger its absolute

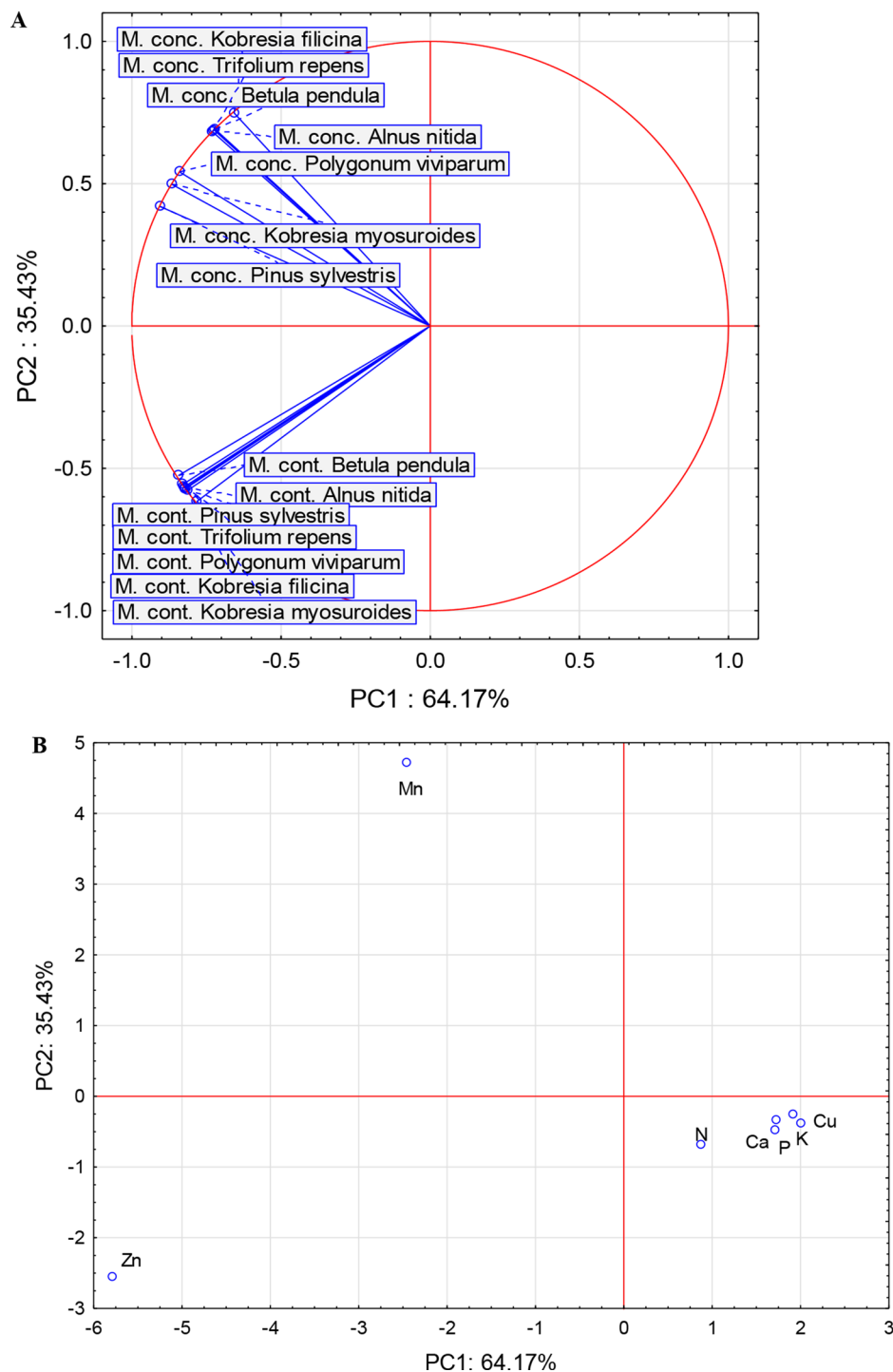


Fig. 4 (A) Projection of variables: parameters on the PC1 and PC2 loadings plot, (B) Projection of sample type on the PC1 and PC2 scores plot

value, the stronger the linear relationship between the variables. The sign of the correlation coefficient indicated a positive or negative correlation between the studied variables. A heat map is a graphical presentation of the data in which the individual values contained in the matrix are represented by a colour scale. The heat map visualized the data through color differences, which can

be seen on the chart as differences, where there were higher values.

Discussion

Kobresia is a small genus of Cyperaceae dominant in the alpine ecosystems, while Polygonaceae ("perennial herbs") are cosmopolitan in northern temperate regions

Table 7 Heat map of the correlation matrix for the tested samples of chemical elements

$r>=$	-1	-0,80	-0,60	-0,40	-0,20	0	0,20	0,40	0,60	0,80	1
	<i>M. conc. Polygonum viviparum</i>	<i>M. cont. Kobresia filicina</i>	<i>M. cont. Kobresia myosuroides</i>	<i>M. cont. Alnus nitida</i>	<i>M. cont. Betula pendula</i>	<i>M. cont. Pinus sylvestris</i>	<i>M. cont. Trifolium repens</i>				
<i>M. conc. Polygonum viviparum</i>	1,000	0,955	0,999	0,980	0,982	0,990	0,982				
<i>M. conc. Kobresia filicina</i>	0,955	1,000	0,939	0,994	0,989	0,905	0,993				
<i>M. conc. Kobresia myosuroides</i>	0,999	0,939	1,000	0,969	0,970	0,996	0,971				
<i>M. conc. Alnus nitida</i>	0,980	0,994	0,969	1,000	0,997	0,944	1,000				
<i>M. conc. Betula pendula</i>	0,982	0,989	0,970	0,997	1,000	0,946	0,997				
<i>M. conc. Pinus sylvestris</i>	0,990	0,905	0,996	0,944	0,946	1,000	0,947				
<i>M. conc. Trifolium repens</i>	0,982	0,993	0,971	1,000	0,997	0,947	1,000				
<i>M. cont. Polygonum viviparum</i>	0,386	0,121	0,432	0,209	0,203	0,509	0,216				
<i>M. cont. Kobresia filicina</i>	0,368	0,110	0,414	0,195	0,184	0,489	0,202				
<i>M. cont. Kobresia myosuroides</i>	0,322	0,061	0,367	0,144	0,137	0,445	0,151				
<i>M. cont. Alnus nitida</i>	0,383	0,111	0,430	0,203	0,199	0,507	0,211				
<i>M. cont. Betula pendula</i>	0,428	0,152	0,473	0,246	0,249	0,549	0,254				
<i>M. cont. Pinus sylvestris</i>	0,380	0,113	0,426	0,201	0,197	0,503	0,209				

of the world. “Anjabar or Maslum”, the common name of *Polygonum viviparum* and easily grow at mountainous territory of Hindu Raj Mountains [29, 30]. Among the herbaceous plant species, the dicotyledenous herb *P. viviparum* and monocot *Kobresia* have typical ECM short roots with sheaths and Hartig nets. The AM (arbuscular mycorrhizal) fungi colonize neither of these plants.

Ascomycete fungi make ectomycorrhizal root tips of the plants [31, 32]. *P. viviparum* and *K. myosuroides* happily associate with ECM fungi [19]. Herbaceous plant growth is severely constrained by the growing season and temperature. As a result, plants create mycotrophic arrangements to maintain the species’ fitness and functionality [33].

The sedge family Cyperaceae, which includes *Kobresia*, is frequently found in stress-inducing environments. Numerous studies have identified the ECM fungal communities of *K. bellardii* [32, 33]. According to Gardes and Dahlberg [34], this is one of the earliest ectomycorrhizal plants to generate the thick pads. To preserve their existence in a non-mycorrhizal condition, tussock-forming sedge quickly absorbs the amino acids [34].

Researchers have investigated the anatomical composition of ECM that develops on the roots of *K. bellardii*. These structures, which demonstrated a mutually beneficial relationship between the plant and fungi, displayed a sophisticated arrangement with clear layers of fungal mantle, Hartig net, and aerenchyma [35, 36].

Dauciform roots are found in sedge species, where limited nutrients and adverse environmental conditions forced the species to make mycorrhizal links. In reaction to phosphate deprivation, Cyperaceae plants (*Schoenus unispiculatus* and *Caustis blakei*) produce dauciform roots [37]. The ECM-colonized roots may be the outcome of an ecophysiological response to alpine adversity, where organisms face the environmental stress. Massicotte et al. [36] described the ECM fungal-based anatomical features of *Kobresia* and *P. viviparum*.

The mycorrhizal status of *Polygonum viviparum* is confirmed [36]. This is an Arctic-alpine herbaceous species of high mountainous regions of the northern hemisphere [38]. Due to several limitations, the mountain compelled the plants to develop symbiotic associations [39]. Ectomycorrhizal root tips of *P. viviparum* comprised Hartig net [32], whereas a few authors reported the absence of ECM fungi in their observations [3, 39]. The lack of variation in the ECM of *P. viviparum* has resulted in a paucity of literature on the existence or absence of ectomycorrhizal structures [40]. Olive-green, pseudoparenchymatous angular mantles of *P. viviparum* and black mycorrhizae lead to their relationship with several trees [3, 41]. Thick mantles enclosed the root apex similarly to the ECM of woody dicotyledonous angiosperms [42]. Ectomycorrhizal and arbuscular mycorrhizal fungal colonization was found in the root system of *P. viviparum* [42–44]. Fontana [44, 45] examined the different fungal morphotypes in *P. viviparum*. Additionally, ectomycorrhizae and ectendomycorrhizae have been documented in *P. viviparum* (a dicotyledonous angiosperm) [46, 47].

Neither Bledsoe et al. nor Adams et al. [3, 41] discovered the ECM in *P. viviparum*. A few *Polygonum* species, like Canadian *P. viviparum*, do not have ECM mycorrhizal association but have dematiaceous surface hyphae. The VAM (vesicular-arbuscular mycorrhizal) is also reported in this species [48]. The ectomycorrhizal linkages in the genera *Kobresia* and *Polygonum* were shown in several research studies, similar to this one [16, 18, 19, 35, 46].

El Omari and El Ghachtouli [49] stated that a multitude of fungal species are extensively employed as bio-control agents in cultivated habitats. The favorable effects of ECM on plant growth are generally acknowledged since it enhances the absorption of water and nutrients, particularly phosphate. Additionally, it provides protection against both biotic and abiotic challenges. Studies of numerous plant species have yielded a wide array of plant responses to colonization. Certain plant species typically do not produce easily identifiable mycorrhizas, most likely because the cost outweighs the benefits. Estimates differ, but research has demonstrated that plants distribute anywhere from 4 to 20% of photo assimilates to mycorrhizal roots. Some species, on the other hand, are facultative mycorrhizal species. Unlike obligatory plants, these species rely on mycorrhizae only when there is a lack of nutrients in the soil.

ECM fungi play a crucial role in forest ecosystems, agriculture, horticulture, biotechnology [50], and environmental sustainability [51]. They have potential applications including biofertilization, biocontrol of plant diseases, and bioremediation of polluted soils [52].

Progress in genomics and molecular biology methods can improve our comprehension of ECM fungal biology, encompassing their genetic variability, evolution, and relationships with host plants and other microbes [53]. ECM fungi contribute to the resilience of forest ecosystems in the face of climate change, helping to reduce its negative effects [54]. The symbiotic relationships between ECM fungus and host plants can optimize the interactions between plants and microbes, leading to improve the functioning of ecosystems [9]. Conducting thorough assessments of ECM fungal diversity and distribution patterns in various biomes and geographical regions will enhance their worldwide biogeography and areas with high biodiversity [55].

Soil is a dynamic biological reactor, where various biochemical reactions and ecological processes occur. The fertility of soil is enhanced by synergistic interactions, competition, and parasitism among microorganisms [56]. Mycorrhiza plays a crucial role in maintaining soil fertility at the global level, addressing significant threats such as soil erosion, soil organic carbon, and nutrient imbalance [57]. Unsustainable land management practices have led to the depletion of soil fertility [58]. ECM has been identified as an eco-friendly approach to improve soil fertility [59]. ECM form a symbiotic relationship that increases the water and nutrient uptake [60].

Mycorrhizal associations can significantly contribute to the decomposition and mineralization of plant organic matter, mobilizing nutrients, particularly nitrogen, for the host plant's benefit. Nitrogen (N) in minerals forms a crucial component of plant growth present in the soil. The plants weakly absorb this, but ECM helps in

mobilization of inorganic nitrogen from the soil [60, 61]. Mycorrhizal roots generate a carbon sink demand, which is amplified by the rise in atmospheric CO₂, hence promoting the growth of ECM. These interactions enhance plant growth, protection, acquiring nutrients, controlling root infections, and plant tolerance to abiotic stressors [62].

Interactions between ECM and other microbes are dependent on various parameters, including the levels of phosphorus and nitrogen in the soil [63]. There is a mutual beneficial link between ECM fungal populations of the soil influenced by nitrogen and phosphorus levels [64]. In this work, the tolerance of fungal species to low soil temperatures (-125 °C) by using a soil mixture with root fragments, mycelium, vegetative mycelium, spores of ECM fungi, and nutrients level were determined. No thresholds of fungal species survival at -125 °C were noted. However, the differences were found in the development and function of mycorrhiza after the exposure at the lowest soil temperature treatments. For frost-tolerant species, the differences as detected in short-term lab treatments reflected the actual differences between the organisms in field conditions too.

The hypothesis was supported by the data regarding the better performance of ECM after very low temperature exposure and clearly observed ECM formation. The ECM supported the plant species *Polygonum viviparum*, *Kobresia filicina*, and *K. myosuroides*. In temperate northern areas, a marked increase in AMF taxa was found in the spring, while the diversity increased in the later growing season. Seasonal changes are attributed to increasing the soil temperature directly or by the availability of carbon from the host [32]. This was remarkable in the study: frost treatments of soil did not influence the proportion of ECM root tips at all. In pure ECM mycelium culture, the growth is even better after exposure to freezing temperatures than unfrozen control, which may be due to minimum competition between the colonies or by activation of mechanisms of physiological recovery after frost [65]. This recovery was favourable for growth and mycorrhizae formation. Similarly, in this experiment during the plant's growth, unlike in nature, the soil temperatures typically remained low in early summer, long after the air temperatures in the growing seasons [66]. Soil temperature of 5 °C in Scots pine resulted in poor root growth, but at 9 °C to 17 °C, ECM formed in all nearby available short roots [67].

Many soluble or exchangeable soil nutrients were found in larger concentrations after the frost exposures. The increased availability was a reflection of foliar nutrients concentration, particularly *K. myosuroides* (Cu, Fe, K, Mn, Zn) [68]. The nitrogen was the only exception, in which both NH₄-N and NO₃-N reduced at all frost treatments. This recommends a change in microbial function

by the freezing treatments. In Maljanen et al. [69] study on spruce forests, decreased soil temperature increased both the concentration and emission of soil NO₃ and N₂O, respectively. Here in this study, NO₃ reduced in the frozen treatments due to leaching from the pots, denitrification, or gaseous 'N' losses. Gaseous 'N' loss and denitrification after freezing in soils is a well-known phenomenon [70]. The possible mechanisms of maximum 'N' availability for denitrifying and other microbes from the litter are disrupted by freeze and thaw, and the competition is reduced for the soluble 'N' by other microbes [71–73]. According to Shane et al. [74], this form of root is morpho-physiologically adapted to grow in nutrient-poor soil and facilitates the plants to get nutrients from the stress-inducing soil.

Here we have shown that ECM of *Polygonum viviparum*, *Kobresia filicina*, and *K. myosuroides* survived in a short exposure of lower temperatures. The soil and foliar nutrient concentrations were determined to assess the direct effects of frost on the soil nutrients and thereby plant nutrition versus nutrient effects caused by changes in the root symbioses. However, the leaf 'N' concentration and content decreased with decreasing temperature due to 'P' deficiency.

Conclusion

In conclusion, there was evidence of better performance of ECM colonization ability after the more severe frost treatments. ECM are more tolerant towards freezing temperatures and affect the availability of soil nutrients for different plant species used in this work of cold-stressed areas.

It may not be possible to determine the mycorrhizal capacity of plants growing in a single soil by just looking at their roots. However, soil and environmental factors influence the mycorrhizal fungal associations in a plant species. The pattern of dispersion of roots within the soil profile and the nutritional conditions are both favourable factors for ECM colonization for plant survival in cold areas.

Future prospects

The potential of ECM fungi is a promising tool to address the difficulties in biotechnology, forestry, agriculture, and environmental conservation. The ecological roles of ECM fungus in soil ecosystems, including their involvement in aggregation, breakdown of organic matter, and cycling of nutrients can also be investigated. ECM fungi can be genetically modified using synthetic biological techniques to tailor them for precise agricultural and environmental uses. There is a chance for fungal populations to shift from ECM to ERM (ericoid mycorrhizal) fungal associations in the near future. Further studies are needed on the topic of the after-effects of different soil

temperatures for a longer time and how the following springtime conditions affect the growth and functioning of ECM.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12866-024-03453-8>.

Supplementary Material 1

Acknowledgements

This research was funded by Researchers Supporting Project number (RSP2024R364), King Saud University, Riyadh, Saudi Arabia.

Author contributions

AU, LD designed the experiments. AU, FA performed the experiments. AU, MAM, MG analyzed and interpreted the data. AU, MAM, FA, wrote and reviewed the manuscript. MG, FAI commented on the manuscript. FA, AU, MAM reviewed and addressed the revision comments with their expertise. All authors read and approved the final version.

Funding

This research received no external funding.

Data availability

The data set generated and analyzed during the current study are available on special request from the corresponding author.

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Institute of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan

²Department of Botany, Faculty of Science, Fayoum University, Fayoum 63514, Egypt

³Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

⁴Department of Chemical Engineering, Qatar University, Doha 2713, Qatar

⁵Laboratoire CHEMBIOPRO (Chimie et Biotechnologie des Produits Naturels), Université de La Réunion, ESIROI Département agroalimentaire, 15 avenue René Cassin, Saint-Denis F-97490, France

⁶Faculty of Production and Power Engineering, University of Agriculture in Krakow, Balicka 116B, Krakow 30 149, Poland

⁷Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, Lublin 20-290, Poland

Received: 9 May 2024 / Accepted: 5 August 2024

Published online: 14 August 2024

References

- Kalucka IL, Jagodziński AM. Ectomycorrhizal Fungi: a major player in early succession. *Mycorrhiza-function, diversity, state of the art*. 2017;187–229.
- Li Y, Xu J, Hu J, Zhang T, Wu X, Yang Y. Arbuscular mycorrhizal fungi and glomalin play a crucial role in soil aggregate stability in Pb-contaminated soil. *Int J Environ Res Public Health*. 2022;19(9):5029.
- Adams F, Reddell P, Webb MJ, Shipton WA. Arbuscular mycorrhizas and ectomycorrhizas on *Eucalyptus grandis* (Myrtaceae) trees and seedlings in native forests of tropical north-eastern Australia. *Austral J Bot*. 2006;54(3):271–81.
- Ruess RW, Hendrick RL, Burton AJ, Pregitzer KS, Sveinbjornsson B, Allen MF, et al. Coupling fine root dynamics with ecosystem carbon cycling in black spruce forests of Interior Alaska. *Ecol Monogr*. 2003;73(4):643–62.
- Tedersoo L, Bahram M, Zobel M. How mycorrhizal associations drive plant population and community biology. *Sci*. 2020;367(6480):eaba1223.
- Rua MA. Characterizing ectomycorrhizal fungal community structure and function of two varieties of *Pinus clausa* that differ in disturbance history. *Forests*. 2021;12(2):219.
- Carteron A, Beigas M, Joly S, Turner BL, Laliberté E. Temperate forests dominated by arbuscular or ectomycorrhizal fungi are characterized by strong shifts from saprotrophic to mycorrhizal fungi with increasing soil depth. *Microb Ecol*. 2021;82(2):377–90.
- Pellitier PT, Ibáñez I, Zak DR, Argiroff WA, Acharya K. Ectomycorrhizal access to organic nitrogen mediates CO₂ fertilization response in a dominant temperate tree. *Nat Commun*. 2021;12(1):5403.
- Chot E, Reddy MS. Role of ectomycorrhizal symbiosis behind the host plants ameliorated tolerance against heavy metal stress. *Front Microbiol*. 2022;13:855473.
- Tedersoo L. Global biogeography and invasions of ectomycorrhizal plants: past, present and future. *Biogeogr Mycorrhizal Symbiosis*. 2017:469–531.
- Dyshko V, Hilszczańska D, Davydenko K, Matic S, Moser WK, Borowik P, et al. An overview of Mycorrhiza in pines: Research, Species, and applications. *Plants*. 2024;13(4):506.
- Li S, Huang X, Shen J, Xu F, Su J. Effects of plant diversity and soil properties on soil fungal community structure with secondary succession in the *Pinus yunnanensis* forest. *Geoderma*. 2020;379:114646.
- Sanz-Benito I, Stadler T, Mediavilla O, Hernández-Rodríguez M, Oria-de-Rueda JA, Dejene T, et al. Into the void: ECM fungal communities involved in the succession from rockroses to oak stands. *Sci Rep*. 2023;13(1):10085.
- Liu Y, Li X, Kou Y. Ectomycorrhizal fungi: participation in nutrient turnover and community assembly pattern in forest ecosystems. *Forests*. 2020;11(4):453.
- Stuart EK, Plett KL. Digging deeper: in search of the mechanisms of carbon and nitrogen exchange in ectomycorrhizal symbioses. *Front Plant Sci*. 2020;10:1658.
- Gao Q, Yang ZL. Diversity and distribution patterns of root-associated fungi on herbaceous plants in alpine meadows of southwestern China. *Mycologia*. 2016;108(2):281–91.
- Kytöviita MM, Ruotsalainen AL. Mycorrhizal benefit in two low arctic herbs increases with increasing temperature. *Am J Bot*. 2007;94(8):1309–15.
- Kagawa A, Fujiyoshi M, Tomita M, Masuzawa T. Mycorrhizal status of alpine plant communities on Mt. Maedake Cirque in the Japan South Alps. *Polar Biosci*. 2006;20:92–102.
- Mühlmann O, Bacher M, Peintner U. *Polygonum viviparum* mycobionts on an alpine primary successional glacier forefront. *Mycorrhiza*. 2008;18:87–95.
- Pasbani B, Salimi A, Aliasgharzad N, Hajiboland R. Colonization with arbuscular mycorrhizal fungi mitigates cold stress through improvement of antioxidant defense and accumulation of protecting molecules in eggplants. *Sci Horticul*. 2020;272:109575.
- Hoch WA, Singaas EL, McCown BH. Resorption protection. Anthocyanins facilitate nutrient recovery in autumn by shielding leaves from potentially damaging light levels. *Plant Physiol*. 2003;133(3):1296–305.
- Meena RS, Kumar S, Rao CS, Kumar A, Lal R. Reforming the soil organic carbon management plans and policies in India. *Plans and policies for Soil Organic Carbon Management in Agriculture*. Springer; 2022. pp. 1–25.
- Morris EK, Morris D, Vogt S, Gleber S, Bigalke M, Wilcke W, et al. Visualizing the dynamics of soil aggregation as affected by arbuscular mycorrhizal fungi. *ISME J*. 2019;13(7):1639–46.
- Martin FM, van Der Heijden MG. The mycorrhizal symbiosis: research frontiers in genomics, ecology, and agricultural application. *New Phytol*. 2024;242(4):1486–506.
- Baker KF. Selective killing of soil microorganisms by aerated steam. Berkeley and Los Angeles: Root Disease and soilborne Pathogens University of California; 1970.
- Kipelaäinen J, Aphalo PJ, Lehto T. Temperature affected the formation of arbuscular mycorrhizas and ectomycorrhizas in *Populus angustifolia* seedlings more than a mild drought. *Soil Bio Biochem*. 2020;146:107798.
- Grace C, Stribley DP. A safer procedure for routine staining of vesicular-arbuscular mycorrhizal fungi. *Mycol Res*. 1991;95(10):1160–2.

28. Harley J, McCreedy uC. The uptake of phosphate by excised mycorrhizal roots of the beech. III. The effect of the fungal sheath on the availability of phosphate to the core. *New Phytol.* 1952;51(3):342–8.
29. Halonen O, Tulkki H, Derome J. Nutrient analysis methods: Metsäntutkimuslaitos; 1983.
30. Malik F, Hussain S, Mirza T, Hameed A, Ahmad S, Riaz H, et al. Screening for antimicrobial activity of thirty-three medicinal plants used in the traditional system of medicine in Pakistan. *J Med Plants Res.* 2011;5(14):3052–60.
31. Nanjundappa A, Bagyaraj DJ, Saxena AK, Kumar M, Chakdar H. Interaction between arbuscular mycorrhizal fungi and *Bacillus* spp. in soil enhancing growth of crop plants. *Fungal Bio Biotechnol.* 2019;6(1):23.
32. Parihar M, Rakshit A, Meena VS, Gupta VK, Rana K, Choudhary M, et al. The potential of arbuscular mycorrhizal fungi in C cycling: a review. *Arch Microbiol.* 2020;202:1581–96.
33. Read D, Haselwandter K. Observations on the mycorrhizal status of some alpine plant communities. *New Phytol.* 1981;88(2):341–52.
34. Gardes M, Dahlberg A. Mycorrhizal diversity in arctic and alpine tundra: an open question. *New Phytol.* 1996;133(1):147–57.
35. Kohn LM, Stasovski E. The mycorrhizal status of plants at Alexandra Fiord, Ellesmere Island, Canada, a high arctic site. *Mycologia.* 1990;82(1):23–35.
36. Massicotte H, Melville L, Peterson R, Luoma D. Anatomical aspects of field ectomycorrhizas on *Polygonum viviparum* (Polygonaceae) and *Kobresia bel-lardii* (Cyperaceae). *Mycorrhiza.* 1998;7:287–92.
37. Playsted CW, Johnston ME, Ramage CM, Edwards DG, Cawthray GR, Lambers H. Functional significance of dauciform roots: exudation of carboxylates and acid phosphatase under phosphorus deficiency in *Caustis Blakei* (Cyperaceae). *New Phytol.* 2006;170(3):491–500.
38. Haselwandter K, Read D. Fungal associations of roots of dominant and sub-dominant plants in high-alpine vegetation systems with special reference to mycorrhiza. *Oecologia.* 1980;45:57–62.
39. Pawłowska S. Charakterystyka statystyczna i elementy flory polskiej [Statistical description and elements of the Polish flora]. W Szafer, K Zarzycki PWN Warszawa [in Polish]. 1972.
40. Dominik T, Niespiak A. The mycotrophy of plant associations in the Dwarf Mountain Pine region of the High Tatra range. 1953.
41. Bledsoe C, Klein P, Bliss L. A survey of mycorrhizal plants on Truelove Lowland, Devon Island, NWT, Canada. *Can J Bot.* 1990;68(9):1848–56.
42. Moreau P-A, Mleczko P, Ronikier M, Ronikier A. Rediscovery of *Alnicola Cholea* (Cortinariaceae): taxonomic revision and description of its mycorrhiza with *Polygonum viviparum* (Polygonaceae). *Mycologia.* 2006;98(3):468–78.
43. Ali H, Muhammad A, Bala NS, Wang G, Chen Z, Peng Z, et al. Genomic evaluations of Wolbachia and mtDNA in the population of coconut hispine beetle, *Brontispa longissima*. *Mol Phylogeny Evol.* 2018;127:1000–9. Coleoptera: Chrysomelidae.
44. Wang G, Zhou Y, Tang B, Ali H, Hou Y. Immune function differences between two color morphs of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) at different life stages. *Ecol Evol.* 2021;11(10):5702–12.
45. Fontana A. editor Ectomycorrhizae in *Polygonum viviparum* L. 3rd North American Conference on Mycorrhizae, Athens, Georgia; 1977.
46. Blaschke H. Multiple mycorrhizal associations of individual calcicole host plants in the alpine grass-heath zone. *Mycorrhiza.* 1991;1:31–4.
47. Treu R, Laursen G, Stephenson S, Landolt J, Densmore R. Mycorrhizae from Denali national park and preserve, Alaska. *Mycorrhiza.* 1995;6:21–9.
48. Li X, Gai J, Cai X, Li X, Christie P, Zhang F, et al. Molecular diversity of arbuscular mycorrhizal fungi associated with two co-occurring perennial plant species on a tibetan altitudinal gradient. *Mycorrhiza.* 2014;24:95–107.
49. El Omari B, El Ghachtouli N. Arbuscular mycorrhizal fungi-weeds interaction in cropping and unmanaged ecosystems: a review. *Symbiosis.* 2021;83(3):279–92.
50. Hu W, Pan L. Applications of mycorrhizal fungi in agriculture and forestry. *Microbial Bioprocesses*: Elsevier; 2023. pp. 1–20.
51. Adnan M, Islam W, Gang L, Chen HY. Advanced research tools for fungal diversity and its impact on forest ecosystem. *Environ Sci Pollut Res.* 2022;29(30):45044–62.
52. Domínguez-Núñez JA. Ectomycorrhizal Fungi as Biofertilizers. *Bioprospects of Macrofungi*. CRC; 2023. pp. 371–83.
53. Naranjo-Ortiz MA, Gabaldón T. Fungal evolution: cellular, genomic and metabolic complexity. *Biol Rev.* 2020;95(5):1198–232.
54. Vincent B, Declerck S. Ectomycorrhizal fungi and trees: brothers in arms in the face of anthropogenic activities and their consequences. *Symbiosis.* 2021;84(3):337–51.
55. Behera AD, Das S. Ecological insights and potential application of marine filamentous fungi in environmental restoration. *Rev Environ Sci Bio/Technol.* 2023;22(2):281–318.
56. Nielsen UN, Ayres E, Wall DH, Bardgett RD. Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity–function relationships. *Eur J Soil Sci.* 2011;62(1):105–16.
57. Fall AF, Nakabonge G, Ssekandi J, Founoune-Mbouh H, Apori SO, Ndiaye A, et al. Roles of arbuscular mycorrhizal fungi on soil fertility: contribution in the improvement of physical, chemical, and biological properties of the soil. *Front Fungal Biol.* 2022;3:723892.
58. Jocien SK, Frederick N. Unsustainable agricultural practices as a driver of soil degradation in Santa sub-division, north west region of Cameroon. *Asian Soil Res J.* 2023;7(2):19–33.
59. Chowdhury SK, Banerjee M, Majumdar T. Chapter-5 The Potential Roles of Mycorrhiza in Sustainable Agriculture: An Eco-Friendly Approach. *Advanced Innovative Technologies in Agricultural Engineering for Sustainable Agriculture.* 2023:59.
60. Basu S, Rabara RC, Negi S. AMF: the future prospect for sustainable agriculture. *Physiol Mol Plant Path.* 2018;102:36–45.
61. Chen A, Gu M, Wang S, Chen J, Xu G, editors. Transport properties and regulatory roles of nitrogen in arbuscular mycorrhizal symbiosis. *Seminars in Cell & Developmental Biology*; 2018.
62. Goicoechea N, Baslam M, Erice G, Irigoyen JJ. Increased photosynthetic acclimation in alfalfa associated with arbuscular mycorrhizal fungi (AMF) and cultivated in greenhouse under elevated CO₂. *J Plant Physiol.* 2014;171(18):1774–81.
63. Hu X, Chen D, Yan F, Zheng X, Fang X, Bai Y, et al. Global research trends on the effects of arbuscular mycorrhizal fungi on the soil carbon cycle: a bibliometric analysis. *Ecol Indic.* 2024;158:111543.
64. Di Sacco A, Hardwick KA, Blakesley D, Brancalion PH, Breman E, Cecilio Rebola L, et al. Ten golden rules for reforestation to optimize carbon sequestration, biodiversity recovery and livelihood benefits. *Glob Change Biol.* 2021;27(7):1328–48.
65. Lehto T, Brosinsky A, Heinonen-Tanski H, Repo T. Freezing tolerance of ectomycorrhizal fungi in pure culture. *Mycorrhiza.* 2008;18:385–92.
66. Domisch T, Finér L, Lehto T, Smolander A. Effect of soil temperature on nutrient allocation and mycorrhizas in scots pine seedlings. *Plant Soil.* 2002;239:173–85.
67. Matzner E, Borken W. Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review. *Eur J Soil Sci.* 2008;59(2):274–84.
68. Zaynab M, Fatima M, Sharif Y, Zafar MH, Ali H, Khan KA. Role of primary metabolites in plant defense against pathogens. *Microb Pathogen.* 2019;137:103728.
69. Maljanen M, Alm J, Martikainen PJ, Repo T. Prolongation of soil frost resulting from reduced snow cover increases nitrous oxide emissions from boreal forest soil. *Boreal Environ Res.* 2010;15:34–42.
70. Yuan W, Rao X, Zhong B, Chen M, Ali H, Lv C, et al. Exploring the functional profiles of odorant binding proteins crucial for sensing key odorants in the new leaves of coconut palms in *Rhynchophorus ferrugineus*. *Int J Biol Macromol.* 2024;261:129852.
71. Wipf D, Krajinski F, van Tuinen D, Recorbet G, Courty PE. Trading on the arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks. *New Phytol.* 2019;223(3):1127–42.
72. Drechsler N, Courty PE, Brulé D, Kunze R. Identification of arbuscular mycorrhiza-inducible nitrate transporter 1/Peptide transporter family (NPF) genes in rice. *Mycorrhiza.* 2018;28:93–100.
73. Xu J, Liu S, Song S, Guo H, Tang J, Yong JW, Ma Y, Chen X. Arbuscular mycorrhizal fungi influence decomposition and the associated soil microbial community under different soil phosphorus availability. *Soil Biol Biochem.* 2018;120:181–90.
74. Shane MW, Cawthray GR, Cramer MD, Kuo J, Lambers H. Specialized 'dauciform' roots of Cyperaceae are structurally distinct, but functionally analogous with 'cluster' roots. *Plant Cell Environ.* 2006;29(10):1989–99.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Terms and Conditions

Springer Nature journal content, brought to you courtesy of Springer Nature Customer Service Center GmbH (“Springer Nature”).

Springer Nature supports a reasonable amount of sharing of research papers by authors, subscribers and authorised users (“Users”), for small-scale personal, non-commercial use provided that all copyright, trade and service marks and other proprietary notices are maintained. By accessing, sharing, receiving or otherwise using the Springer Nature journal content you agree to these terms of use (“Terms”). For these purposes, Springer Nature considers academic use (by researchers and students) to be non-commercial.

These Terms are supplementary and will apply in addition to any applicable website terms and conditions, a relevant site licence or a personal subscription. These Terms will prevail over any conflict or ambiguity with regards to the relevant terms, a site licence or a personal subscription (to the extent of the conflict or ambiguity only). For Creative Commons-licensed articles, the terms of the Creative Commons license used will apply.

We collect and use personal data to provide access to the Springer Nature journal content. We may also use these personal data internally within ResearchGate and Springer Nature and as agreed share it, in an anonymised way, for purposes of tracking, analysis and reporting. We will not otherwise disclose your personal data outside the ResearchGate or the Springer Nature group of companies unless we have your permission as detailed in the Privacy Policy.

While Users may use the Springer Nature journal content for small scale, personal non-commercial use, it is important to note that Users may not:

1. use such content for the purpose of providing other users with access on a regular or large scale basis or as a means to circumvent access control;
2. use such content where to do so would be considered a criminal or statutory offence in any jurisdiction, or gives rise to civil liability, or is otherwise unlawful;
3. falsely or misleadingly imply or suggest endorsement, approval, sponsorship, or association unless explicitly agreed to by Springer Nature in writing;
4. use bots or other automated methods to access the content or redirect messages
5. override any security feature or exclusionary protocol; or
6. share the content in order to create substitute for Springer Nature products or services or a systematic database of Springer Nature journal content.

In line with the restriction against commercial use, Springer Nature does not permit the creation of a product or service that creates revenue, royalties, rent or income from our content or its inclusion as part of a paid for service or for other commercial gain. Springer Nature journal content cannot be used for inter-library loans and librarians may not upload Springer Nature journal content on a large scale into their, or any other, institutional repository.

These terms of use are reviewed regularly and may be amended at any time. Springer Nature is not obligated to publish any information or content on this website and may remove it or features or functionality at our sole discretion, at any time with or without notice. Springer Nature may revoke this licence to you at any time and remove access to any copies of the Springer Nature journal content which have been saved.

To the fullest extent permitted by law, Springer Nature makes no warranties, representations or guarantees to Users, either express or implied with respect to the Springer nature journal content and all parties disclaim and waive any implied warranties or warranties imposed by law, including merchantability or fitness for any particular purpose.

Please note that these rights do not automatically extend to content, data or other material published by Springer Nature that may be licensed from third parties.

If you would like to use or distribute our Springer Nature journal content to a wider audience or on a regular basis or in any other manner not expressly permitted by these Terms, please contact Springer Nature at

onlineservice@springernature.com