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Running title: Niche differentiation between life-history stages

Competition for resources is ameliorated by niche differentiation between *Solidago virgaurea* life-history stages in the Arctic

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Abstract

Aims: Competition has been shown to modify the niche breadth of coexisting species, but within species interactions have received little attention. Establishing small juvenile individuals and established, larger, sexually reproducing adult individuals represent two life-history stages within species. We investigated the nitrogen and carbon resource use of adult and juvenile individuals and similarity of symbiotic fungal community composition in these two plant life stages. We used the plant *Solidago virgaurea* growing in a simplified system in the low Arctic as model species.

Methods: Isotopic signatures (foliar $\delta^{15}N$ and foliar $\delta^{13}C$) were analysed to characterise nitrogen acquisition and water-use efficiency of the plants. Symbiotic root fungal community composition was estimated by cloning and sequencing small subunit rRNA gene.

Important findings: The isotopic signatures differed significantly between the life stages, indicating that the establishing juvenile cohort used relatively more amino acids or gained N through mycorrhizal symbiosis in comparison to the established adult plants. Symbiotic fungal communities did not differ between the two plant cohorts suggesting a possibility that the plants shared the same mycorrhizal network. We conclude that competition mediated differences in plant resource use may create niche differentiation between the two life-history stages and enable them to coexist.

Key words: competition, low Arctic, niche, resource-use, stable isotope natural abundance

Introduction

The search for the determinants of plant community assembly has remained so far unresolved although various models and approaches have been constructed during the last 150 years. While these approaches certainly capture many of the essential elements of species coexistence, the complexity and the multitude of the interactions seems to defy the emergence of a general theory of plant coexistence. It is clear that issues related to the arrival on a site (dispersal) and thriving on a site (competitive ability) are essential ingredients of any successful strategy. In this paper, we focus on the elements of intraspecific interactions in plant-community dynamics.

Intraspecific competition influences the density-dependence of growth and plant population characteristics (Begon et al. 2006) and it is generally considered stronger than interspecific competition because individuals of the same species have the same resource requirements and the same resource-obtaining structures (Tilman 1982). However, there is also evidence that among all plants (intra- and interspecific) competition for nutrients and light is mainly a result of size inequality rather than due to species-specific trait differences (Goldberg and Barton 1992). Most plant species create local aggregations generated by clonal growth, limited seed dispersal and patchy environments (Stoll and Prati 2001). As a result, plant individuals interact mainly with close, often conspecific neighbours, increasing the importance of intraspecific mechanisms affecting plant communities (Antonovics and Levin 1980; Pacala 1997).

Aboveground competition involves mainly competition for light whereas belowground competition includes competition for available soil resources. Plants may avoid belowground resource competition by niche differentiation, e.g. through variation between species in rooting depth (Parrish and Bazzaz 1976; Berendse 1979; Mamolos et al. 1995) or differential use of nutrient forms (McKane et al. 2002; Miller and Bowman 2002; von Felten et al. 2009). Nitrogen (N) is often a limiting resource in Arctic ecosystems (McKane et al. 2002) and available in different chemical forms (Kielland 1994; McKane et al. 2002). The most available form of nitrogen in the Arctic tundra is organic N, specifically amino acids (Kielland 1994), but ammonium availability may also be significant (Nordin et al. 2004) especially in areas intensively grazed by megaherbivores. Plant species have been shown to differ in their ability to take up organic and inorganic forms of N (Miller and Bowman 2002) and the availability of different N forms in soils may thus be an important element in determining the distribution of species. In response to changes in the accessibility of chemical forms of N, plant species may show plasticity in the uptake of N form and/or shift preference from one N form to another. Coexisting plant species of strongly N-limited Arctic tundra have been shown to differ in N use with dominant plant species using the most available forms of N and subordinate species using alternative N forms, thus facilitating species coexistence (McKane et al. 2002). In a tracer-experiment by McKane et al. (2002), five plant species in Arctic tussock tundra community occupied different niches in terms of use of ammonium, nitrate and glycine (amino acid) and species dominance strongly correlated with the uptake of most accessible soil nitrogen forms. Ashton et al. (2010) discovered resource use plasticity by the dominant species to facilitate species coexistence in alpine dry meadow. However, the authors did not find the rarer

species to switch its N preference in competitive environment (Ashton et al. 2010). Although classic theory of species coexistence emphasises the role of niche differences, theoretical work suggests that competitive outcomes may be determined by the combined effects of both; species niche differences and differences in their competitive ability (Chesson 2000; Adler et al. 2007; Mayfield and Levine 2010).

In addition to competition, facilitative interactions may play an important role in regulating the composition of plant communities (Callaway 1995; Fajardo and McIntire 2011). Facilitation can be defined as positive interaction between plants where one plant enhances the growth, survival or reproduction of a neighbour (Callaway 2010). The core idea of the Stress Gradient Hypothesis (Bertness and Callaway 1994) is that under low levels of abiotic stress, competition dominates whereas under harsh conditions, facilitation prevails over competition. However, if abiotic conditions become very extreme, facilitation may diminish (Maestre et al. 2009). Most evidence of positive interactions comes from stressful environments, for example from Arctic, alpine, desert and saltmarsh systems (Callaway 2007; Brooker et al. 2008). A stressful environment may favour intraspecific facilitative net effects (Goldenheim et al. 2008; Fajardo and McIntire 2011), although the evidence is mixed (Eränen and Kozlov 2008).

Seedlings receive both facilitative and competitive effects from neighbouring adult plants. The benefits may result from refuge from thermal stress by provision of shelter, elevated soil nutrient availability by litter fall or accumulation of debris (Franco and Nobel 1988; Nobel 1989) and physical protection from herbivory (Callaway 1995). On the other hand, seedlings may suffer from increased competition for light, soil water or nutrients by

the competitively superior adult plants (Franco and Nobel 1988; Nobel 1989; Holmgren et al. 1997). The outcome of interactions is the net effect of both positive and negative plant–plant interactions (Pugnaire and Luque 2001). Although classically examined at interspecific level, competition and facilitation may also occur at the intraspecific level (Fajardo and McIntire 2011).

Arbuscular mycorrhizas (AM) have been shown to mediate belowground interactions between plants (Hart et al. 2003; van der Heijden et al. 2003). The obligatory symbiotic AM fungi explore the soil farther than plant roots, assimilate nutrients and transfer these to the host plant in exchange for carbon (Smith and Read 2008). The AM fungi and plant roots are able to utilise the same N pools (ammonium and amino acids) (Govindarajulu et al. 2005; Whiteside et al. 2012). The benefit of the symbiosis is thought to arise from faster and more cost efficient nutrient assimilation in mycorrhizal roots (Tuomi et al. 2001; Smith et al. 2009). The AM symbiont is one of the major sinks of carbon for the host and it has been estimated that about 20-30% of plant photosynthates are consumed by the AM symbiosis (Smith and Read 2008). The net benefit of AM symbiosis to the plant may vary from negative to positive, depending on the symbiont identities and environmental conditions (Klironomos 2003). In addition to direct nutrient acquisition effects, plant roots in natural communities are often linked by common AM mycelial network (Simard and Durall 2004; Selosse et al. 2006). Common mycorrhizal networks (CMN) can mediate distribution of soil resources between plants and affect plant-plant interactions, but information regarding their exact effect is conflicting (Moora and Zobel 2010). It has been suggested that by connecting to a mycorrhizal network seedlings acquire

easier access to soil resources and CMN may benefit seedling establishment and growth (Grime et al. 1987; van der Heijden 2004). CMN may represent a strategy for substantial resource saving to seedlings as they are able to connect to a mycorrhizal network supported by photosynthates from older, larger plants (van der Heijden 2004). However, if plant size affects sink strength for the shared resources in the CMN, the nutrient benefits may be unevenly distributed (Kytöviita et al. 2003) and the seedlings may grow less in the presence of an established network (Moora and Zobel 1996; Pietikäinen et al. 2007; Janouskova et al. 2011).

The measurement of the stable isotope composition of nitrogen and carbon may provide insights into resource dynamics in plants. Foliar $\delta^{15}N$ signatures have been used as a natural tracer to investigate plant access to different N sources (Högberg 1997; Robinson 2001). There is great variability in isotopic composition of soil N pools available to plants and ammonium, nitrate and organic N may differ in their isotopic compositions (Robinson 2001) suggesting that foliar $\delta^{15}N$ signal may reflect the plant available N in the soil (Houlton et al. 2007). However, source identification is complicated by isotopic fractionation, which occurs during physiological processes and biological mechanisms that discriminate against the heavier ^{15}N in favor of the lighter ^{14}N (Dawson et al. 2002). For example, mycorrhizal fungi discriminate against the heavier ^{15}N isotope during transfer of N from the fungus to the host plant, leaving the plant depleted in ^{15}N (i.e. with a more negative $\delta^{15}N$ value) (Hobbie and Hobbie 2008; Hobbie and Högberg 2012). Foliar $\delta^{13}C$ is commonly used to determine plant water-use efficiency and identify the photosynthetic pathway (Farquhar et al. 1989; Dawson et al. 2002). The $\delta^{13}C$ ratio is the result of

discrimination against the heavier $^{13}CO_2$ during diffusion through the stomata and resulting carboxylation in favor of the lighter $^{12}CO_2$ (Farquhar et al. 1989). Environmental stress, such as drought, may restrain CO_2 diffusion into leaves and decrease the internal partial pressure of CO_2 below normal, resulting in foliage less depleted in ^{13}C (Farquhar et al. 1989). Since stomatal conductance is relational to water loss, $\delta^{13}C$ is positively correlated with water-use efficiency (Farquhar and Richards 1984). Spatial and temporal variability in environmental conditions (e.g., light, temperature, humidity, soil water) can influence stomatal conductance and contribute to differences in foliar $\delta^{13}C$ in terrestrial vegetation (Garten and Taylor 1992).

In our present work, we hypothesize that due to intraspecific competition juvenile plants acquire different nitrogen sources than conspecific adults. If so, juveniles and later plant life stages represent a case of niche differentiation during life-history. We used isotopic signatures (foliar $\delta^{15}N$ and foliar $\delta^{13}C$) to characterise nitrogen acquisition strategy and water-use efficiency in two different life stages of a common herbaceous species. We assess the potential networking effect of the AM symbionts and their relationship to host resource acquisition by measuring the similarity of the AM community in the roots of the two plant life stages. Similarity or dissimilarity of fungal community has been used previously as a measure to assess networking potential (i.e. Aldrich-Wolfe 2007; Kennedy et al. 2012). The intensity of belowground competition for resources is difficult to reveal under field conditions, therefore we established a unique experimental setup. We created field plots with simplified vegetation in low Arctic meadow, which allowed evaluation of

intraspecific adult plant - juvenile interactions. The results elucidate mechanisms of resource competition in Arctic plant communities critical to recruitment and coexistence.

Materials and methods

Study species

Solidago virgaurea (L.) is an herbaceous perennial species in the Asteraceae family. It has a broad distribution in the northern hemisphere (Hulten and Fries 1986) and it is one of the preferred forage species by reindeer (Skogland 1980). *S. virgaurea* is a very common species in low Arctic habitats. The frequency of AM in roots of *S. virgaurea* growing in Arctic meadows is over 90% (Kytöviita et al. 2011). It forms a rosette of leaves and one or several floral shoots which reach the height of 15 to 30 cm in the Arctic. *S. virgaurea* reproduces mainly by seeds and clonal growth is very limited (database of clonal growth in plants; Klimešová and de Bello 2009). Even though seeds are wind dispersed, the majority of seeds germinate in the vicinity of the mother plant.

Site and sampling

This study was conducted in two low Arctic meadows in Kilpisjärvi, north-west Finland. The vegetation is mainly composed of the grasses *Deschampsia flexuosa* and *Festuca ovina* with underlying moss cover consisting of *Hylocomium splendens* and *Pleurozium schreberi*. The most common herbs are *Solidago virgaurea*, *Bistorta vivipara*,

Saussurea alpina and *Trollius europaea*. The amount of above ground plant biomass is about 500 g m^{-2} (Pietikäinen et al. 2005). The organic layer of the soil in the area is ca. 6 cm thick and the soil temperature measured at 3–5 cm depth during July and August (warmest months), is on average 11.2 °C (2005–2011).

We used study plots with experimentally simplified vegetation in two sites; Jehkas (69°05′N, 20°47′E) and Saana (69°03′N, 20°50′E) (Kytöviita et al. 2011). The vegetation was simplified by removing all vegetation in the plots (3.5 m in diameter) in 1999 and transferring 100 adult *S. virgaurea* plants from the surrounding meadow to each plot (6 plots in total). The plots were covered with fine mesh from mid-August to early June until 2005 to prevent seed rain. After the growing season 2005, natural seed rain was allowed to reach the plots. At the beginning of the experiment 1999 the vegetation in the plots consisted solely of *S. virgaurea*. The dominant plant species in the study plots the year leaves were collected (2011) was still *S. virgaurea*, but *Deschampsia flexuosa*, *D. cespitosa* and *Festuca ovina* were present. The dominance of *S. virgaurea* and scarcity of other plants resulted in a competitively simplified environment in comparison to a natural meadow. To describe the intensity of intraspecific competition we explored the densities of *S. virgaurea* seedlings in the study plots, which were high when recorded a year after the mesh had been removed (in 2006) with on average 7 seedlings per 10 cm^2 with mean seedling shoot dry weight 3.9 mg (n = 36). Samples for calculating seedling density and biomass in the plots were taken with soil core (diameter 6.0 cm). No *S. virgaurea* established from seed had reached the size required for sexual reproduction by 2011, whereas the planted adult *S. virgaurea* flowered annually already two years after planting.

In July 2011, we collected leaf and root samples from the two ontogenetic stages: the plants that were planted in 1999 and from juvenile individuals that had germinated on the plots after 2005. We call these plants adult and juvenile hereafter. The juvenile plants (shoot dry weight on average 12.75 ± 2.26 mg) were growing within 30 cm of an adult plant. The juvenile plants were small (<5cm shoot height), had several leaves and no cotyledons and thus were at least two years of age, but less than five as establishment was possible only since 2005. The transplanted adult plant age is unknown, but as they were already flowering year 1999, their age is considerably greater than 12 years. The size difference between the adults and the juveniles was distinct as the size of a juvenile plant (shoot + roots) was approximately the same as one adult plant leaf.

From 30 adults (flowering, over 50 rosette leaves), leaves and roots were collected from five individuals per plot (two sites, three plots per site). Five fully grown leaves of similar size per plant were randomly collected to make one sample. Root samples were collected from the same individuals. For the juvenile plants, 30 composite leaf and root samples were collected in the same way. However, as the juvenile plants were small, to obtain five samples per plot that contained enough leaf and root material for analyses, we combined all leaves and roots from 4-8 juveniles to make one sample. It is possible that the leaf age can affect the foliar $\delta^{15}N$ values (Domenach et al. 1989). *S. virgaurea*, however, is a deciduous plant producing new leaves every spring. Therefore, the leaves that we collected from the adults and the juveniles were of similar age.

We measured root density and rooting depth of *S. virgaurea* in the study plots by collecting horizontal soil cores at depths 0–10 and 10-25 cm (n = 33) to determine the

distribution of root mass in the soil profile. The cores (diameter 3.0 cm) were taken below adult *S. virgaurea* plants. The soil cores were individually sieved using 2 mm sieve and all roots were collected. Sieved soil was collected for soil $\delta^{15}N$ analysis to define the isotopic pattern present in the soil.

Analyses

Stable isotopic signatures Leaf samples were dried (65°C, 24h) and ground to a fine powder in a ball mill. Powdered samples 1.5 mg each, were placed into foil capsules (Elemental Microanalysis, D1008, BN225926) and sealed. Soil samples were dried (30°C, 48h) and sieved using 125µm ø sieve. Soil samples weighing 6 mg each, were also sealed in foil capsules. Measurements of total N and C and stable isotope ratios of N and C were conducted using an elemental analyser (Flash EA1112, Carlo Erba) connected to a Finnigan Deltaplus Advantage (Thermo Electron Corp., Waltham, USA) continuous flow isotope ratio mass spectrometer (CFIRMS). Natural abundances of ^{15}N and ^{13}C are expressed in per mil (‰) deviation from international standards: $\delta^{15}N$ or $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000$, where R is the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Atmospheric nitrogen and Pee Dee Belemnite were used as the international standards for N and C.

Root weight density Roots were carefully washed with copious water and dried at 30°C 24h before weighing. Root weight density (RWD) was calculated as total dry weight of roots per volume (mg/cm^3).

AM community analysis Root samples for fungal DNA analysis were taken from all the study plants (adults n = 30, juveniles n = 30 composite samples). Total DNA was

extracted from root dry weight material (0.2 g) using a NucleoSpin Plant II Kit following the manufacturer's recommendations (Macherey-Nagel). Partial small-subunit (SSU) ribosomal RNA gene fragments were amplified using nested PCR with the universal eukaryotic primers NS1 and NS4 in the first reaction and specific fungal primers AML1 and AML2 in the second reaction (Lee et al. 2008). The use of SSU rRNA is commonly used method in AM community studies (Öpik et al. 2013; 2014). The PCR reactions were carried out using the same method as Francini et al. (2014). The reaction yields were estimated on agarose gel. After the second PCR the products were pooled per plot. Pooling was done separately for adults and juveniles. After pooling, the number of samples for cloning was 12. The pooled samples were purified with QIAquick PCR purification kit (Qiagen) and cloned using InsTAclone PCR Cloning Kit (Thermo Scientific) and transformed into *Escherichia coli* (JM109). Positive transformants were amplified using M13 forward and reverse primers, with the following cycling conditions: 95 ºC for 3 min, 95 ºC 30 s, 55 ºC 30 s, 72 ºC 60 s, (32X) and the final extension 10 min at 72 ºC. Product quality and size were screened in agarose gels and at least 16 positive clones from each sample were sequenced on ABIPrism 3130 x 116 (Applied Biosystems) automated sequencer using M13 forward primer and BigDye Terminator v3.1sequencing kit (Promega). Sequences were edited manually using BioEdit 7.2.5. Edited sequences were compared to the public database in GenBank using BLAST (Altschul et al. 1990) to exclude any possible plant sequences and to the virtual taxa (VTX) listed in the Maarj*AM* database (Öpik et al. 2010). We found six plant sequences and excluded them from the analysis. A cutoff level of 97% was used in group definition. Alignment of the sequences and phylogenetic analysis were conducted using MEGA version 6. The sequences derived

from the Maarj*AM* database were included in the phylogenetic analysis as references. Bootstrap values were calculated with 1000 replicates and the evolutionary distances were computed using the Tamura-3 parameter method. Sequences have been deposited in GenBank under accession numbers KU361597 – KU361788.

Statistical analyses Foliar and soil data were analysed using three-way nested ANOVA using Levene's test to confirm equality of variances. In the analyses, values of $\delta^{15}N$, $\delta^{13}C$, foliar nitrogen concentration (N%), root weight density (RWD), soil $\delta^{15}N$ and soil N% were used as dependent variables. Site (Jehkas, Saana) and plant life stage (adult or juvenile) or soil depth (0-10 cm or 10-25 cm) were used as independent fixed factors and plot as a random factor. In the analysis the plot was nested within the site. Root weight density was lg10 transformed to satisfy the normality assumptions of the test. Correlation between foliar $\delta^{15}N$ and N% and between $\delta^{15}N$ and AM community was tested with Pearson correlation (r_p). The REGR factor scores in the $\delta^{15}N$ and AM community correlation were obtained from 18 individual OTUs. Normality of the data was checked through the normality of residuals. Analyses were done using IBM SPSS Statistics 20 for Windows.

The effect of site and plant life stage on AM community structure was tested by using Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson 2001). PERMANOVA was carried out using site and size as a fixed factors. To visualize the differences in the AM community composition, principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarities was used. Analysis was conducted in PRIMER software v6 (Clarke and Warwick 2001). Mothur 1.36.1 software was used to estimate rarefaction

curves for the number of operational taxonomic units (OTUs) in order to characterize sampling adequacy of the AM sequences obtained.

Results

Supporting our initial hypothesis, the *S. virgaurea* juveniles had significantly more negative $\delta^{15}N$ and $\delta^{13}C$ values compared to adults (Table 1, Fig. 1). Overall, plants had slightly more negative $\delta^{13}C$ signal in Jehkas compared to Saana (Fig. 1). The site did not have a main effect on $\delta^{15}N$ values. However, there was a significant interaction between site and life stage of the plant (Table 1). The adults had higher $\delta^{15}N$ in Saana than Jehkas whereas the site did not affect $\delta^{15}N$ in the juveniles.

The study site and life stage (adult, juvenile) had a significant effect on foliar N concentration (N%) (Table 1). The foliar N% was higher in Saana than Jehkas ([Saana: 2.68 ± 0.08, n= 29] [(Jehkas: 2.06 ± 0.06, n=30]) and the juveniles had slightly lower foliar N% than the adult plants ([adult: 2.49± 0.09, n=30] [juvenile: 2.25± 0.08, n=29]). There were no interactions in foliar N% and the relationship between foliar N% and ^{15}N enrichment was weak ($r^2 = 0.102$) ($r_p = 0.318$, n = 59, $P = 0.014$). Soil N% differed significantly between the sites (Table 2): it was higher in Saana compared to Jehkas site (Table 3).

Root weight density varied significantly with soil depth (Table 2) and most of the *S. virgaurea* roots were concentrated within the upper layer of soil (Table 3). The $\delta^{15}N$ value of the soil was not significantly affected by plot, site or soil depth (Tables 2 and 3).

The AM community in the roots did not differ significantly between the adults and the juveniles (Table 4, Fig. 2). However, the AM community structure in *S. virgaurea* was clearly different between the Jehkas and Saana sites. Neighbour-joining (Fig. 3) analyses and comparison with sequences in the MaarjAM database of 192 glomalean SSU sequences obtained from *S. virgaurea* root samples matched to 15 different VTXs in the MaarjAM database. Three of them; VTX00030 (i.e. *Acaulospora sp.*), VTX00028 (i.e. *Acaulospora sp.*) and VTX00149 (i.e. *Glomus Glo G5, Glomus Alguacil12b GLO G11*) were confined to plants in Jehkas and three; VTX00005 (i.e. *Archaeospora sp.*), VTX00009 (i.e. *Archaeospora Desiro13a MIB 8531*) and VTX00357 (i.e. *Claroideoglomus Torrecillas12b Glo G8*) to adult plants in Saana. All phylogenetic groups had VTX match. To address how well the AM sequencing data represented the actual mycorrhizal community, rarefaction curves of alpha diversity were built for OTUs formed at the 97% similarity level. We found 18 different OTUs. As shown in Fig. 4, rarefaction curves did not reach saturation in juvenile plant sequences from Jehkas but were beginning to flatten in all the other groups where the sampling captured most of the diversity. There was no correlation between foliar mean $\delta^{15}N$ and root AM community ($r^2 = 0.190$) ($r_p = -0.435$, n = 12, $P = 0.157$) (Fig. 1S).

Discussion

Differences in tissue ^{15}N and ^{13}C contents are classically used to delineate species niche, trophic position and resource use in ecological studies (Dawson et al. 2002; Post 2002). In this work, we show that within the same species, two life-history stages can differentiate in terms of N acquisition strategy. The long-time measurements in the present work and those in Kytöviita et al. (2011) show that juvenile *S. virgaurea* compete intensively with established adult *S. virgaurea.* Between species, competition is considered to result in niche differentiation (Parrish and Bazzaz 1976; Tilman 1982; Fargione et al. 2003). Here we show that the same principle may apply to a within species scenario.

Niche differentiation may take place during plant life-history due to environmental conditions that favour early life-history stages that are different from those favouring later stages (Callaway and Walker 1997; Soliveres et al. 2010). It is conceived that seedling recruitment may take place in a regeneration niche (Grubb 1977), the young plants persist in a persistence niche (Bond and Midgley 2001) and successful sexual reproduction may require a reproductive niche (Bykova et al. 2012). In the present case, the juvenile plants are likely to have been competitively limited into a persistence niche by the established adult *S. virgaurea*. This is in line with our earlier work where the presence of adult *S. virgaurea* reduced conspecific seedling growth (Kytöviita et al. 2011). The juvenile shoot masses from 2006 and 2011, indicate that the juveniles remain very small (in average <13 mg) in the study plots in the presence of adult *S. virgaurea*. In comparison, in the absence

of adult plants the juveniles reach over ten times more aboveground biomass within two years (Kytöviita et al. 2011).

Foliar $\delta^{15}N$ signatures result from different factors including access to different N sources and the effects of isotopic fractionation (Högberg 1997; Robinson 2001). Further variability in foliar $\delta^{15}N$ values may be caused by plant rooting depth as the soil $\delta^{15}N$ varies with depth (Hobbie and Ouimette 2009; but see Chang and Handley 2000), typically by becoming enriched in deeper layers (Hobbie and Ouimette 2009). However, in the Arctic the soil organic layer is generally very shallow (Tarnocai and Campbell 2002); in the present experimental vegetation type about 6 cm (Stark and Kytöviita 2006) and roots are concentrated in the top soil (Jackson et al. 1996). In the current study, most roots of *S. virgaurea* were found within the upper 10 cm of soil and the plots had uniform vertical $\delta^{15}N$ distribution, which allowed us to exclude the possibility of rooting depth effects on plant $\delta^{15}N$ signal.

The leaves of juvenile *S. virgaurea* had more negative $\delta^{15}N$ value than the adult plants indicating that the plants received N from a different source, thus reducing the resource competition by niche differentiation. Soil organic nitrogen pools are isotopically lighter (containing less ^{15}N) than inorganic pools of nitrogen (Yano et al. 2010; but see Hobbie and Högberg 2012). The lower $\delta^{15}N$ in the juveniles could indicate that the juveniles utilised relatively more amino acids when compared to adult plants. In Arctic ecosystems, the presence of mycorrhizal symbioses in most plant species complicates the studies of pathways for nutrient partitioning between plants. Alternative to directly using organic vs inorganic N sources, the lower $\delta^{15}N$ in juvenile plant leaves may be explained by

the greater contribution of mycorrhizal fungi in their N acquisition. AM fungi have been shown to take up inorganic N (NO_3^- or NH_4^+) (Govindarajulu et al. 2005) and organic N (amino acids) (Näsholm et al. 1998; Whiteside et al. 2012) and transfer them to their host plant. When transferring N to host plants mycorrhizal fungi fractionate against ^{15}N (Hobbie and Hobbie 2008; Hobbie and Högberg 2012) which results in $\delta^{15}N$ depletion in the host plant (i.e. with a more negative $\delta^{15}N$ value). The seedlings in the study site are intensively mycorrhizal (Kytöviita et al. 2011), which means that mycorrizal effect on resource partition is likely to be strong and that the AM may be an important part of the persistence niche. This agrees well with the common notion that the mycorrhiza may be more important to plants in the early life stages (van der Heijden 2004).

The AM fungal communities did not differ between the adult and the juvenile *S. virgaurea* hosts. In terms of OTUs obtained, 94% were observed in both adult and juvenile plants, and only five were specific to adult and six to juvenile plants. Several authors have proposed that seedlings are typically colonized by an existing mycelial network (Newman 1988; Kytöviita et al. 2003; van der Heijden 2004). Since adults and juveniles in the experiment hosted the same AM community, it is likely that the juveniles were connected to an existing mycelial network, but to verify this, other type of studies (CMN manipulation experiments in container systems) are required. The more negative $\delta^{15}N$ in the juveniles indicates that they may have received N resources from mycorrhiza. However, regardless of the mycorrhiza-derived nitrogen, the juveniles in our study sites have remained very small during the time that plant growth has been followed in the study sites (6 years). These results from field are consistent with a greenhouse experiment where Arctic plants

connected to mycorrhizal network were rapidly colonized by arbuscular mycorrhiza (Varga and Kytöviita 2016), but gained no growth benefits in longer term (Kytöviita et al. 2011). Therefore, it seems that CMN does not facilitate juvenile transition from persistence to reproduction within Arctic plant community.

Water acquisition and desiccation avoidance strategies differ between plant species and are part of species-specific niche dimensions (Silvertown et al. 2014). We used foliar $\delta^{13}C$ to estimate plant water economy which is commonly used to determine plant water-use efficiency and water status (Farquhar et al. 1989; Dawson et al. 2002). In the present study, foliar $\delta^{13}C$ values were higher in the adults than in the juveniles at both study sites. Plants experiencing drought stress typically reduce their stomatal openings to decrease the rate of water loss and this elevates their $\delta^{13}C$ values (Farquhar et al. 1989). In Arctic tundra, taller plants are more exposed to wind, which could result in adult *S. virgaurea* experiencing more drought stress and therefore having higher $\delta^{13}C$ values when compared to the juveniles. Thus given that the adult plants showed the most enriched values of $\delta^{13}C$, the results imply that adult plants had the greatest water-use efficiency and as such, fixed the most carbon per unit amount of water transpired. Generally, large neighbouring plants can affect juvenile performance in their presence by adjusting microclimatic conditions such as light, temperature and soil humidity (Holmgren et al. 1997; Bruno et al. 2003). In the current study, the $\delta^{13}C$ results indicate that the juveniles growing in the shelter of adult plants were experiencing less water stress and that adult plants may have facilitated the juvenile *S. virgaurea* in terms of water economy. Facilitation is considered particularly important in vegetation dynamics under harsh environmental conditions (Bertness and

Callaway 1994; Callaway and Walker 1997; Holmgren et al. 1997). The effects of mycorrhizal fungi on plant responses to drought stress have been discussed (Smith and Read 2008); however, it is challenging to dissociate nutritional effects from direct effects on water transport since the hyphal input to nutrient uptake becomes more significant as soil dries (Finlay 2008). Carbon isotopic measurements have showed that AMF are capable to enhance stomatal conductance in plants and stimulate photosynthetic capacity and WUE (Querejeta et al. 2006). Plants may further transfer hydraulically lifted water directly to the mycelia of their mycorrhizal fungi (Querejeta et al. 2003), and mycelia can then redistribute hydraulically lifted water to multiple plants connected to the same CMN (Egerton-Warburton et al. 2007; Allen 2009). In the current study, it is possible that the AMF aided the plant water economy to some degree; however, water was limiting adult plant performance at least periodically in the harsh Arctic environment.

In conclusion, this study supports the view that persistence niche may segregate from reproduction niche in terms of nitrogen source utilization in the Arctic. Although interspecific differences in N source have been previously documented (Michelsen et al. 1996; McKane et al. 2002; Miller and Bowman 2002), this is the first examining intraspecific niche differentiation. The scarcity of previous work may be explained by the need for long-term manipulations in order to obtain simplified systems that allow within species ontogenetic differences to be evaluated. We conclude that plasticity in resource use and niche dimensions could be an important mechanism for coexistence of different plant generations that warrants future research.

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Tables

Table 1. ANOVA table showing df, MS, F and P values of the response variable $\delta^{13}C$, $\delta^{15}N$ and N% in the plant species *Solidago virgaurea*. The three factors are the site (Saana, Jehkas), life stage of the plant (adult, juvenile) and plot nested within site.

$\delta^{13}C$	df	MS	F	*P*
Site	1	5.945	15.646	**0.017**
Life stage	1	3.820	7.246	**0.010**
Plot(Site)	4	0.380	0.721	0.582
Site*Life stage	1	0.194	0.368	0.547
Error	51	0.527		
$\delta^{15}N$				
Site	1	3.197	1.284	0.321
Life stage	1	69.370	146.301	**<0.001**
Plot(Site)	4	2.493	5.258	**0.001**
Site*Life stage	1	3.568	7.524	**0.008**
Error	51	0.474		
N%				
Site	1	5.661	41.088	**0.003**
Life stage	1	0.744	6.329	**0.015**
Plot(Site)	4	0.138	1.172	0.334
Site*Life stage	1	0.001	0.004	0.948
Error	51	0.118		

Table 2. ANOVA table showing df, MS, F and P values of the response variable root weight density (RWD: mg root dry weight /cm^3 soil), soil $\delta^{15}N$ and soil N% in *Solidago virgaurea*. The three factors are the site (Saana, Jehkas), depth (1-10 cm and 10-25 cm) and plot nested within site.

RWD	df	MS	F	*P*
Site	1	0.351	7.380	0.113
Depth	1	2.145	18.234	**<0.001**
Plot(Site)	2	0.048	0.404	0.672
Site*Depth	1	0.294	2.498	0.126
Error	27	0.118		

Soil δ15N	df	MS	F	*P*
Site	1	3.747	6.299	0.129
Depth	1	0.311	1.387	0.249
Plot(Site)	2	0.595	2.654	0.089
Site*Depth	1	0.061	0.274	0.605
Error	27	0.224		

Soil N%	df	MS	F	*P*
Site	1	0.103	21.030	**0.044**
Depth	1	0.000	0.115	0.737
Plot(Site)	2	0.005	4.253	**0.025**
Site*Depth	1	0.001	0.855	0.363
Error	27	0.001		

Table 3. Mean root weight density (RWD: mg root dry weight /cm^3 soil), soil $\delta^{15}N$ and soil N% in Saana and Jehkas sites.

		RWD (mg/cm^3)		Soil $\delta^{15}N$		Soil N%		
	Depth	Mean	s.e.	Mean	s.e.	Mean	s.e.	*n*
Saana	0-10 cm	0.85	0.22	4.71	0.23	0.32	0.01	10
	10-25 cm	0.36	0.06	4.90	0.20	0.20	0.02	7
Jehkas	0-10 cm	0.73	0.10	4.07	0.08	0.08	0.01	8
	10-25 cm	0.20	0.05	4.18	0.07	0.07	0.01	8

Table 4. PERMANOVA results for AM community composition showing df, MS, F and P values. The factors are site (Saana, Jehkas) and life stage of *Solidago virgaurea* (adult, juvenile).

Parameters	df	MS	Pseudo-F	P(MC)
Site	1	3537.9	4.3235	**0.014**
Life stage	1	212.32	0.2594	0.875
Site*Size	1	1202.6	1.4697	0.23
Residual	8	818.28		
Total	11			

Figures

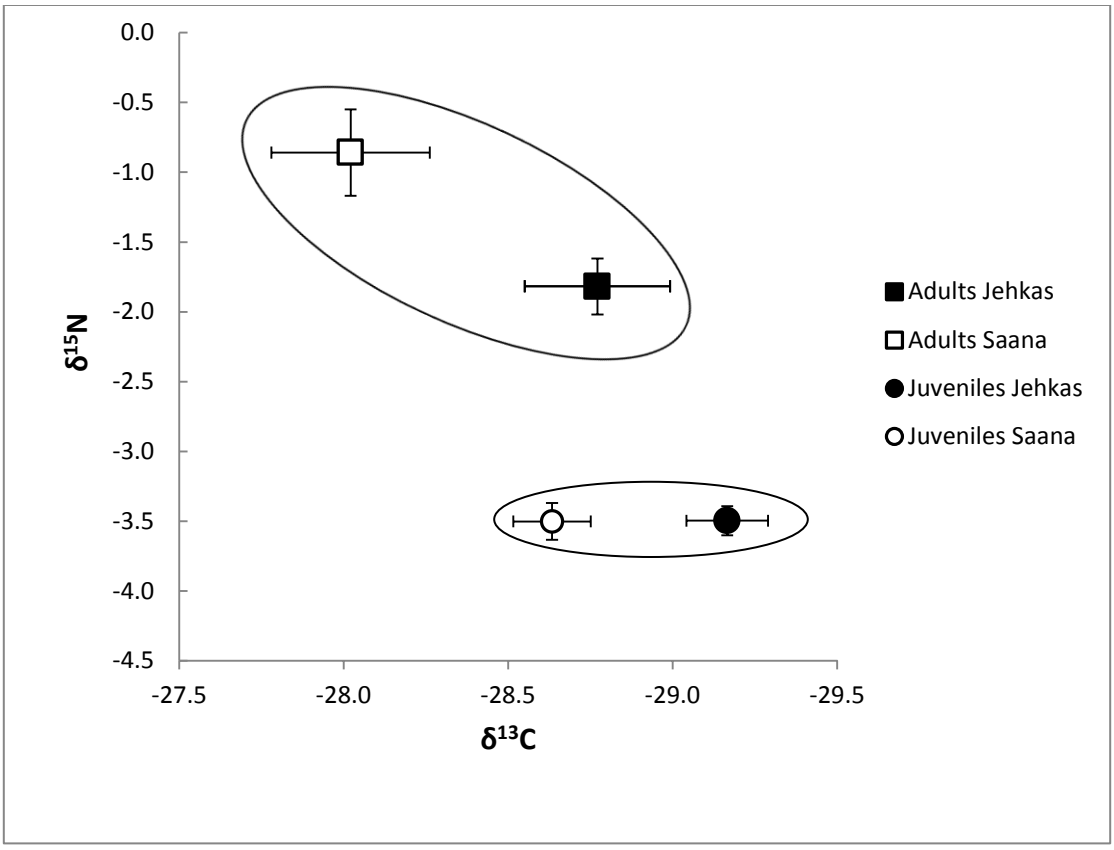

Figure 1. Mean foliar values (± s.e.) of $\delta^{15}N$ and $\delta^{13}C$ in adult and juvenile *Solidago virgaurea* at the Jehkas and Saana sites.

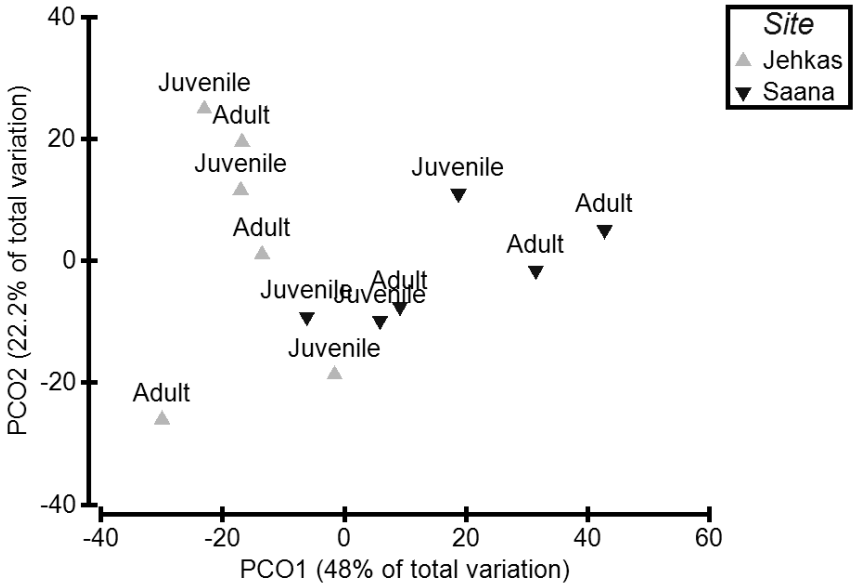

Figure 2. Principal-coordinate analysis (PCoA) based on Bray-Curtis dissimilarity of arbuscular mycorrhizal community structure in the roots of adult and juvenile *Solidago virgaurea* at the Jehkas and Saana sites. OTUs were classified at 97% similarity level.

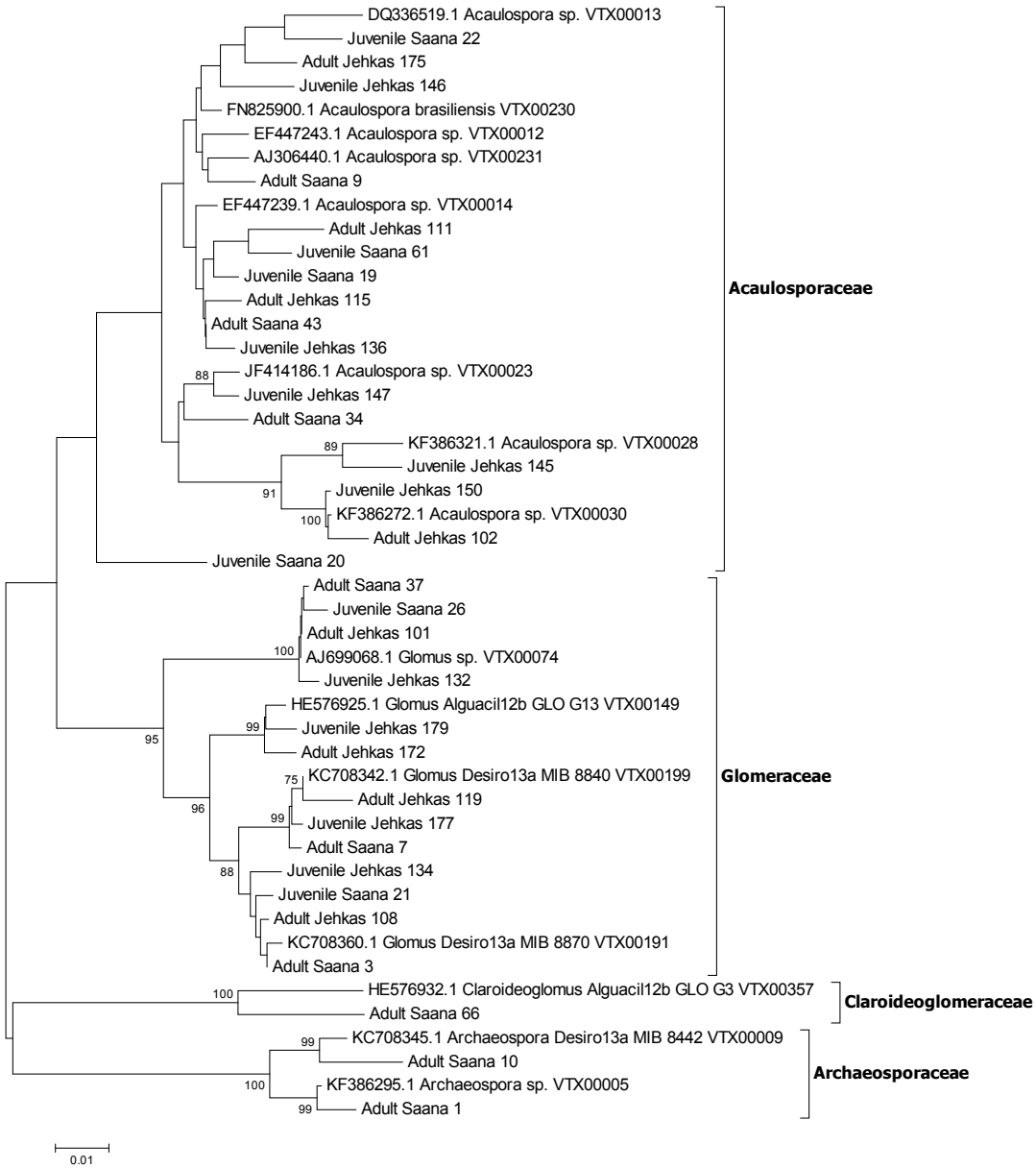

Figure 3. Neighbour-joining phylogenetic tree inferred from partial SSU rRNA gene sequences of AM fungi in adult and juvenile *Solidago virgaurea* at the Jehkas and Saana sites and reference sequences. Tree was constructed with a set of sequences representative of the 15 VTXs (one representative per group [Adult Jehkas, Adult Saana, Juvenile Jehkas,

Juvenile Saana]). Group identity and clone number are shown for the representative sequences. Bootstrap values (1,000 replicates) >70 % are presented. *S*equences retrieved from Maarj*AM* database are used as the reference group, and reported here with GenBank accession numbers and VTX numbers. All sequences have been submitted to the GenBank database under accession numbers KU361597 – KU361788.

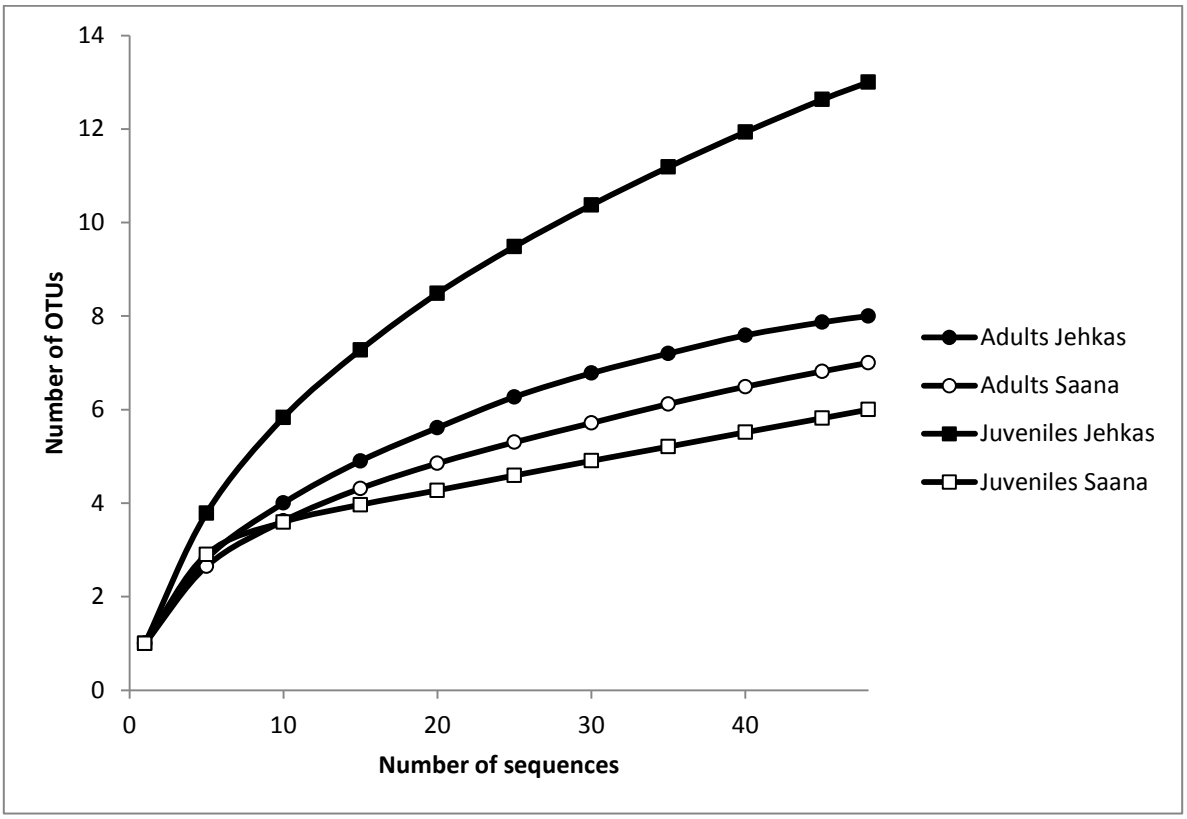

Figure 4. Rarefaction curves representing arbuscular mycorrhizal operational taxonomic units (OTUs) of the small subunit rRNA gene vs number of sequences of adult and juvenile *Solidago virgaurea* at the Jehkas and Saana sites.