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A new perspective on arctic soil metazoan diversity:
metagenetics reveals local and geographical patterns
of variation in community structure and habitat
specialization in high arctic tundra of Svalbard

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A new perspective on arctic soil metazoan diversity: metagenetics reveals local and geographical patterns of variation in community structure and habitat specialization in high arctic tundra of Svalbard

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ABSTRACT

Little is known of the diversity, community structuring, niche differentiation and habitat specialization of small soil Metazoa in polar environments.

Here, I studied three contrasting high arctic tundra types at Kongsford, NW Svalbard (78° 55' N), comparing the small soil Metazoa community in each along with the comparison to a mid-latitude temperate forest site in Korea (37 deg.N), using an identical interrupted grid sampling scheme. In addition, communities of nematode present in local microsites (rhizosphere, cyanobacterial mat, etc.) in the arctic tundra were also compared.

Soil Metazoa, mostly nematodes were extracted using combined Baermann funnel and sugar flotation, and the DNA extracted, PCR amplified for the NF1-18Sr2b region of the 18s rRNA gene, and 454 pyrosequenced.

Our samples revealed diverse communities of soil Metazoa in all three tundra types, with species proxy (operational taxonomic unit, [OTU]) diversity far exceeding the species diversity based on morphological surveys in previous studies of Svalbard. There was no difference in OTU α-diversity between the three tundra types. I found no correlation between nematode and soil properties but across individual samples there was a positive correlation between

Shannon α-diversity with TOC, C/N ratio and P2O5. β-diversity was significantly higher in IV and LV tundra, suggesting that their mosaic of bare and vegetated patches supports a greater range of local metazoan communities than the more uniformly vegetated HV tundra.

HV tundra had a distinct community from the LV tundra type, with the community of IV tundra falling between these in terms of OTU composition, indicating an important element of niche and habitat differentiation amongst small soil Metazoa between the three different tundra types.

Different microsite types were differentiated at some degree based on NMDS resulted from Bray Curtis similarity matrix. The strongest differences were between rhizophere and cyanobacterial mat areas, and this pattern was consistent for Nematoda and for all Metazoa combined. However, no distinct community composition of the Metazoa was found within the microsites (e.g. between the rhizosphere of two different cushion plant species) suggesting the limits of microhabitat specialization in this environment.

Overall, total nitrogen, total organic carbon and available phosphorus in the soil in each microsite were the best predictor of variation in both total metazoan and nematode communities. Despite the evidence of niche specialization in the communities, there was

only about 5.56% of overlap in OTUs shared among different microsites suggesting that many species are actually quite generalized in their distribution and most likely in their ecology.

However, I concluded that despite this being an 'extreme' environment amongst land ecosystems, normally thought to require generalized niches amongst animals, the Metazoa in the high arctic tundra are still to some extent habitat-specialized. When the Svalbard tundra was compared with temperate forest, arctic tundra had markedly lower alpha-diversity for soil Metazoa than the temperate forest, reinforcing the view that there may be a 'classic' latitudinal diversity difference in this group. However, two of the three sites in the Svalbard tundra had higher beta diversity than the Korean temperate forest, while a third tundra site has equally high beta diversity. This may reflect the greater influence of small scale environmental heterogeneity within the tundra compared to temperate forest.

Also of interest is the fact that while most Metazoa OTUs in the temperate forest did not occur in the tundra, and vice versa, reflecting the degree of geographical endemism or environmental specialization that differentiates these regions. However, a small proportion (around 10%) of species do apparently occur in both environments despite their very distinct environments.

Keywords: nematode, DNA, tundra, metagenetics, 454 pyrosequencing, diversity.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

to sample the greatest range of taxa of soil Metazoa.

Fig. 7: Vector of environmental variables added NMDS based on Bray-Curtis of total Metazoa (left) and Nematoda (right) of different microsites. Stress value measures the deviation of departure from monotonicity of the original n-dimensional space and distance in the reduced k-dimensional ordination space (McCune and Grace, 2002). C.Mat stands for cyanobacterial mat.

Fig. 8: NMDS of soil properties based on Euclidean distance.

Fig. 9: Putative percentage relative abundance (by reads) of nematode feeding groups in different microsites, based on numbers of reads falling into each category. C.Mat stands for cyanobacterial mat.

Fig. 10: NMDS of Community composition of rhzosphere of 2 plant species (Carex parallela, Silene acaulis) and cyanobacterial mat (represented as C.Mat) with environmental variables included.

Fig. 11: Interrupted grid sampling system used in this study.

Fig. 12: 12 OTU richness of soil Metazoa and nematode in different

locations.

Fig. 13: β-diversity of Metazoa in arctic tundra and temperate biome.

Fig. 14: NMDS based on UniFrac distance of metazoan community of temperate biome and arctic tundra.

Fig. 15: NMDS based on UniFrac distance of nematode community of temperate biome and arctic tundra.

Fig. 16: Shared OTUs of all metazoans compared between the arctic (Svalbard Tundra) and temperate region (Korean forest). The 5 temperate region samples harbor a much higher number of metazoan OTUs (species) than the 5 arctic samples, and only 35 OTUs are shared between the two regions.

Fig. 17: Shared OTU (species) of nematode compared between temperate (Korean forest) and arctic (IV tundra) region. The 5 temperate region samples harbor more than twice the number of species than 5 arctic region samples and only 19 species are overlapped.

CHAPTER 1. GENERAL INTRODUCTION

1.1 General ecology of arctic soil Metazoa

One of the central aims of ecology is to understand the patterns in community composition and diversity of living organisms in nature, and from these to understand the still poorly-understood mechanisms which underlie them.

The polar environments of northern Svalbard, close to the KOPRI Dasan Ny-Alesund Base, offer a view of life close to its limits. One of the greatest challenges in understanding polar ecosystems is to understand how the patterns of diversity and community composition of organisms adjust themselves to local microclimates, and differences in the availability of resources. As well as giving better understanding of the survival and coexistence of different forms of life, this may also help explain the seasonal and spatial patterns of biogeochemical processes of the tundra. Also of great interest in ecology is the broader scale question of how the

extreme climate and limited resources of the polar environment alter

community structure, niche width and diversity in comparison to other

ecosystems in warmer climates. Through such comparisons, general

theories of community structure and species coexistence may be arrived at.

Over the years, there has been a considerable amount of study of

plants and larger animals (from large mammals, down to insect size) in

polar environments, including Svalbard (e.g. Alsos et al. 2012). In contrast,

very little attention has been given to local and regional diversity trends in

small soil Metazoa (roughly <0.1－2mm in length) in the high Arctic and

Antarctic (Coulson 2013) (often now referred to as soil 'meiofauna', a

term borrowed from marine ecology), even though these may be a

significant part of the diversity, a large part of the biomass, and perform

important functions within the ecosystem (Coulson 2013). This is

understandable because many of these organisms are very small, often

only visible through a microscope or magnifying glass, and hard to

distinguish from one another because of their morphological similarities.

Nematodes as an example of soil Metazoa. Polar nematodes appear to have even less studied than most other soil invertebrate groups (Coulson 2013). Even though they are considered to be the most abundant animals on Earth, they have often been completely ignored in community and food web studies, even when many other small animals were included (Hodkinson & Coulson 2004). In general, the nematode ecology of polar regions also appears to be the most poorly understood of any terrestrial system, as many more studies have been done at boreal, temperate and tropical latitudes in both moist climate ecosystems and deserts (e.g. Shepard et al. 2002). This bias against nematodes in general, and polar nematodes in particular, is at least partly due to the practical difficulties of sampling and identifying nematodes in remote locations using traditional morphological criteria under a microscope. Of the few previous studies which have focused on tundra or polar desert nematode community distribution and diversity, the work by Kennedy (1993), Powers et al. (1995), Treonis et al. (1999) and Porazinska et al. (2002) in the dry

valleys of Antarctica revealed a low overall diversity of nematodes (often 1-3 species in each microhabitat), but quite distinct communities adapted to particular substrates and microclimates, often separated by just a few meters. In the polar semi-desert of Devon Island, Canada, distribution of nematodes also depended very much on microsite (Cockell et al. 2001).

'Micro-oases' of greater plant cover associated with nutrient concentrations, and with high populations of soil bacteria and fungi, had greater abundance and diversity of nematodes. In all of these studies, nematode diversity, while restricted to only a few species, was higher in warmer, moister and more nutrient-rich microsites. An exception is the study by Mouratov et al. (2001) on King George Island, just off the Antarctic Peninsula, where the sparse tundra/polar semi desert also yielded no more than 3 or 4 nematode species in most samples, but abundance and diversity was lower in the dampest microsites – perhaps because of the generally moister soil conditions at this locality, giving some waterlogged and low nutrient peaty soils.

On Svalbard, only 8 nematode genera were identified in a study by Klekowski & Opalinski (1986) on tundra at Fugelberget at the southern end of the archipelago. As in most polar studies, nematode abundance and diversity was concentrated into areas of greater moisture, vegetation cover and organic matter content. Despite their low apparent diversity, the potentially key importance of nematodes as decomposers and detritivores, and mineralizers of N and P, was recognized in the authors' description of the Svalbard tundra ecosystem. For Svalbard as a whole, 113 nematode species have been recorded through morphological identification, including both soil and shallow freshwater nematodes (Coulson 2013), although Coulson (2013) emphasizes that on Svalbard soil invertebrates including nematodes remain relatively poorly studied.

It is unclear how the diversity and guild structure of polar nematode communities compares with those of lower latitudes. Boag & Yeates (1998) suggested that the global peak of soil nematode diversity lies not in the tropics but between 30 and 40 degrees N or S, in the mid latitudes, and

reaches its lowest point in the high latitudes above 70 degrees N and S. That conclusion was at the time based on only two studies from the Arctic, and several from Antarctica.

It is also thought that the guild structure of polar nematode communities may be much simpler than in warmer climates. Studies from Antarctica's dry valleys and King George's Island reveal that plant feeders and specialized predators are mostly or entirely absent, and that the main nematode species present are bacterial feeders and omnivore (Mouratov et al. 2001).

Up until recently all studies of nematodes, such as those cited above, relied on morphological criteria. However, in the past several years has it become possible to assess biodiversity of soil metazoans rapidly by bulk physical isolation of the organisms from soil and extraction of their DNA en masse. Massively parallel sequencing of selected marker genes allows taxonomic classification and estimation of diversity and relative abundances (Porazinska et al. 2009). In their pioneering studies,

Porazinska et al. (2009, 2010, 2012) demonstrated the feasibility of using bulk isolation followed by DNA extraction and 454-pyrosequencing of the small soil animal community of soils. They found that a large number of reads of both mites and nematodes were obtained (Porazinska et al. 2010).

The metagenetic studies of Porazinska et al. (2012) showed that around half of all tropical and temperate soil nematodes are bacterial/fungal feeders, with plant parasites and predators also being predominant. This differs from the picture from classical morphological criteria (Mouratov et al, see above). There is a need to reinvestigate the guild structure of polar nematodes using rigorous and standardized metagenetic criteria that can give a more conclusive answer.

Mites and other small soil metazoa. Much that can be said of nematodes in polar environments is also true of other soil Metazoa, including mites (Acari, members of the Arthropoda), and such phyla as Tardigrada, Platyhelminthes, smaller Annelida, Gastroticha, etc., all of which are known to be widely present in soils. Mites in particular are

known to be abundant in soils everywhere (Shepard et al. 2002), but their diversity and community composition is poorly characterized in polar environments, because of their small size and the difficulty of identification. A few polar studies have, however, been carried out – all of them using classical morphological methods. Hodkinson et al. (2004) and Hodkinson & Coulson (2004) studied small soil arthropods including mites, Collembola and insect larvae along a glacier foreland succession on Svalbard. They found that soil arthropod diversity increased along the chronosequence, with repeated, deterministic changes in communities likely linked to increasing plant diversity. They found that food web complexity was greater than usually imagined, with high percentages of parasitoids, predators, and hyperparasitoids. Treonis et al (1999) studied Antarctic dry valleys, and found a low overall diversity of mites and other non-nematode groups, with diversity and abundance strongly linked to moisture availability in the soil.

It is unclear how metagenetic methods would change the overall

picture of the diversity and community structure of non-nematode soil

Metazoa. The studies of Porazinska et al. have shown that nearly half the

metazoan reads, and around 20% of species, obtained from soils in tropical

and temperate rainforest were from these non-nematode groups. Our own

analyses also showed strong representation of various groups, especially

Acari in both Korea and Malaysia. Even before the advent of soil

metagenetics, it was widely suggested that no more than 8% of the true

diversity of mite species in the world had already been discovered

(Shepard et al. 2002). Clearly, there is considerable potential for a new

perspective on the soil Metazoa. So far, no detailed ecological analyses

have been carried out in any part of the world using metagenetics on these

other non-nematode Metazoa - but the potential is there, if the same

procedures for classification following online published reference

sequences are followed. It is possible that the DNA-based perspective will

reveal the true extent and patterns in diversity on Svalbard of these

groups where so many unknown/cryptic species are likely to be present.

The ease and rapidity of the metagenetic method would also certainly facilitate more extensive ecological study of soil Metazoa than has been done so far.

1.2 Objectives of this study

The present study was structured around investigating the following questions:

1. To what extent are soil metazoan communities of <0.1−2mm in length (hereafter referred simply as soil Metazoa) differentiated between different tundra types on Svalbard? Is there a difference in soil Metazoa community composition, and α − and β − diversity, between the different main tundra types of Svalbard, and in relation to environmental variables? Which environmental variables have a stronger role in structuring community composition?

2. How does the overall diversity of soil metazoan in Svalbard tundra detected by this methodology compare to that found in studies using morphological criteria?

3. Are soil metazoan communities in different microsites (amongst plant roots, under moss clumps, on open cyanobacterial mat areas, and polygon) distinct from one another and thus indicating niche differentiation among different environments? If indeed they are distinct, what soil parameters best predict such distinct soil metazoan communities within the Svalbard tundra?

4. Is the soil metazoan community specialized enough to be distinct between the root systems of different species of tundra plants?

5. Is there evidence of a strong latitudinal difference in soil Metazoa alpha- and beta- diversity between the high arctic (Svalbard) and the temperate zone (Korea), paralleling what is found for many other groups across wide latitudinal differences? I predicted that Svalbard would have much lower alpha and beta diversity, in line with what is found for most other groups of organisms , such as trees, birds and mammals (Lyons and Willig, 1999)

6. Is there evidence of a strong degree of species overlap between the

high arctic and temperate zone, reflecting highly generalized niches

in small soil Metazoas? Each species has a definable ecological

range in terms of climate and geography (Dumbrell et al., 2009;

Vandermeer, 1972). For larger organisms such as plants and

mammals, there is little or no overlap in terms of the species

present between high arctic and lowland temperate environments –

the exception being birds which have definite summer-winter

migration patterns between the two environments (Johnson and

Herter, 1990). I predicted that due to the very different

environmental requirements for survival in temperate and polar

regions, there would be little or no evidence of species overlap.

7. Is there a difference in trophic guild structure of soil Metazoas

between Svalbard and Korea, possible reflecting fundamental

differences in community and ecosystem functioning? It has long

been thought that in warmer, moist climates, with greater primary

productivity and less extreme physical conditions relative to the

optimum temperatures for cellular processes, specialized feeders on

larger living organisms (e.g. parasites, specialized predators, top

predators) will be more diverse and abundant (Pianka, 1966). In

extreme environments with low productivity, it is supposed that

resource supply is less predictable, preventing specialization by

predators and parasites, and food web structure is likely to be

simpler with fewer specialized parasites and predators.

CHAPTER2. DISTINCT SOIL METAZOAN COMMUNITIES ACROSS DIFFERENT TUNDRA TYPES WITHIN THE SVALBARD HIGH ARCTIC TUNDRA.

2.1 Introduction

Over the past 150 years there has been a considerable amount of study of community patterns of plants and larger animals — from large mammals, down to insect size — in polar environments of the arctic and Antarctic (e.g. Alsos et al., 2012; Jónsdóttir, 2005). In contrast, very little attention has been given to community structure and diversity trends of small soil Metazoa (roughly <0.1–2mm in length) in the high arctic (Coulson, 2013) even though these may be a significant part of the overall diversity, a large part of the biomass, and also perform important functions within the ecosystem (Bongers and Ferris, 1999; De Deyn et al., 2003; Lavelle et al., 2006). For instance, it has been suggested that micro–arthropods play an

important role in decomposition and formation of arctic soil, as there are often low abundances of annelids and macroinvertebrates (Coulson et al., 2000; Sørensen et al., 2005). However, little is known about the ecological role nematodes and other small Metazoa, such as annelids, tardigrades, arthropods etc. have on arctic environment.

Several studies of small Metazoa on Svalbard have been carried out since the 1980s. Only eight nematode genera were identified in a study by Klekowski & Opalinski (1986) on tundra at Fugelberget at the southern end of the archipelago. As in other polar studies (Bölter et al., 1997; Cockell et al., 2001; Yergeau et al., 2007), they found that nematode abundance and diversity was concentrated into areas of greater moisture, vegetation cover and organic matter content. For Svalbard as a whole, 113 nematode species have been recorded through morphological identification, including both soil and shallow freshwater nematodes (Coulson, 2012). This is much lower than the totals for other warmer parts of the world (Boag and Yeates, 1998; Lawton et al., 1996; Yeates, 1999).

Other Metazoa on Svalbard also showed lower diversity compared to other regions of the world, although many studies were not directly comparable. Coulson (2012) found 36 species of Annelida, 89 species of Tardigrada, and 152 species of Acari on Svalbard. By contrast, BASSET and Kitching (1991) found 795 species of microarthropods from two years of research in an Australian rainforest tree. In the case of Tardigrada, 380 species were found in an extensive set of surveys across the Americas (North America, South America, Central America and the West Indies) including terrestrial and freshwater environments (Meyer, 2013). In a south Florida estuary, 44 species of polychaetous annelids were identified (Santos and Simon, 1974). The lack of strict comparability in methods makes it impossible to judge if Svalbard is in fact less diverse in these groups than other parts of the world.

Until recently, all ecological studies of small soil Metazoa, such as those cited above, relied on morphological criteria. However, in the past several years it has become possible to assess biodiversity of soil Metazoa

1 6

by bulk physical isolation of the organisms from soil and extraction of their DNA en masse. Massively parallel sequencing of selected marker genes allows taxonomic classification and estimation of diversity and relative abundances (Porazinska et al., 2009). In their pioneering studies, Porazinska et al. (2009, 2010, and 2012) demonstrated the feasibility of using bulk isolation of metazoan bodies followed by DNA extraction and 454-pyrosequencing of the small animal community in soils. They found that a large number of reads of both Acari and Nematoda were obtained, and experiments with artificial assemblages of species showed that the abundance of reads approximately reflected the abundance of each species in soil (Porazinska et al., 2010).

Not only have these molecular-based methods greatly facilitated rapid sampling, they have also revealed a much greater 'hidden' nematode diversity than was suspected from morphological studies. (Fonseca et al., 2010). For instance, Fonseca et al., (2010) identified 182 nematode species in a temperate benthic ecosystem using a metagenetic approach;

the time spent on the research was only 3% of that required by a parallel study using the traditional morphological method which identified 113 nematode species after three years (Lambshead, 1986).

The present study aimed to answer the following questions: To what extent are soil metazoan communities of <0.1−2mm in length (hereafter referred simply as soil Metazoa) differentiated between different tundra types on Svalbard? Is there a difference in soil Metazoa community composition, and α − and β − diversity, between the different main tundra types of Svalbard, and in relation to environmental variables? Which environmental variables have a stronger role in structuring community composition? How does the overall diversity of soil metazoan in Svalbard tundra detected by this methodology compare to that found in studies using morphological criteria?

2.2 Methodology

2.2.1 Site description.

Fieldwork was conducted in Svalbard in late July, 2013, at sites within reach of the Dasan Base, at 78° 55′ N in north-western Svalbard (Fig. 1). I concentrated on sampling the main tundra types in the area, designated on the basis of percentage vegetation cover as follows:

1. The "high vegetation cover tundra" (HV) has over 90% of vegetation cover; is located several meters above sea level near the coastline, above the level of the highest tides near Vestre Lovenbreen (Haldorsen and Heim, 1999) (latitude 78° 55′ 20.0″ N, longitude 11° 56′ 30.3″ E), and was mostly covered by various bryophytes, the dwarf willow *Salix polaris* Wahlenb and lichens.

2. The "intermediate vegetation cover tundra" (IV), has vegetation cover between 70 to 85%. It was located on slightly raised terraces,

a few hundred meters inland near the CCT (Climate Change Tower) (latitude 78° 55′ 18.55″ N, longitude 11° 51′ 52.63″ E). A previous study described this site as patchy vegetation in which the dominant plant species were *Dryas octopetala* and bryophytes, along with a Luzula/lichen heath zone (Coulson et al., 2000). During the time I sampled, the site was covered mostly by bryophytes and *Salix polaris* Wahlenb. Previous research on a similar location (78° 55 N, 11° 53 E; Coulson et al., 2000) found the area dry and well−drained without glacial meltwater or groundwater input.

3. The "low vegetation cover tundra" (LV) showed more bare patches and vegetation cover of less than 50%, accompanied by a much higher abundance of arctic polygons. The site is described by other authors as semi polar desert and previous studies showed *Dryas octopetala* as a dominant species in this site (Welker et al., 1993; Wookey et al., 1993). I found *Salix polaris* Wahlenb and *Carex fuliginosa* as dominant species in our quadrats, along with less moss

20

cover than in other sites. Also, by visual examination, the surface of

the soil in this zone (latitude 78° 55′ 58″ N, longitude 11° 49′

22″ E) had less coverage of small stones and organic debris,

compared to HV tundra. This tundra type was present on raised

banks and terraces more than several hundred meters inland.
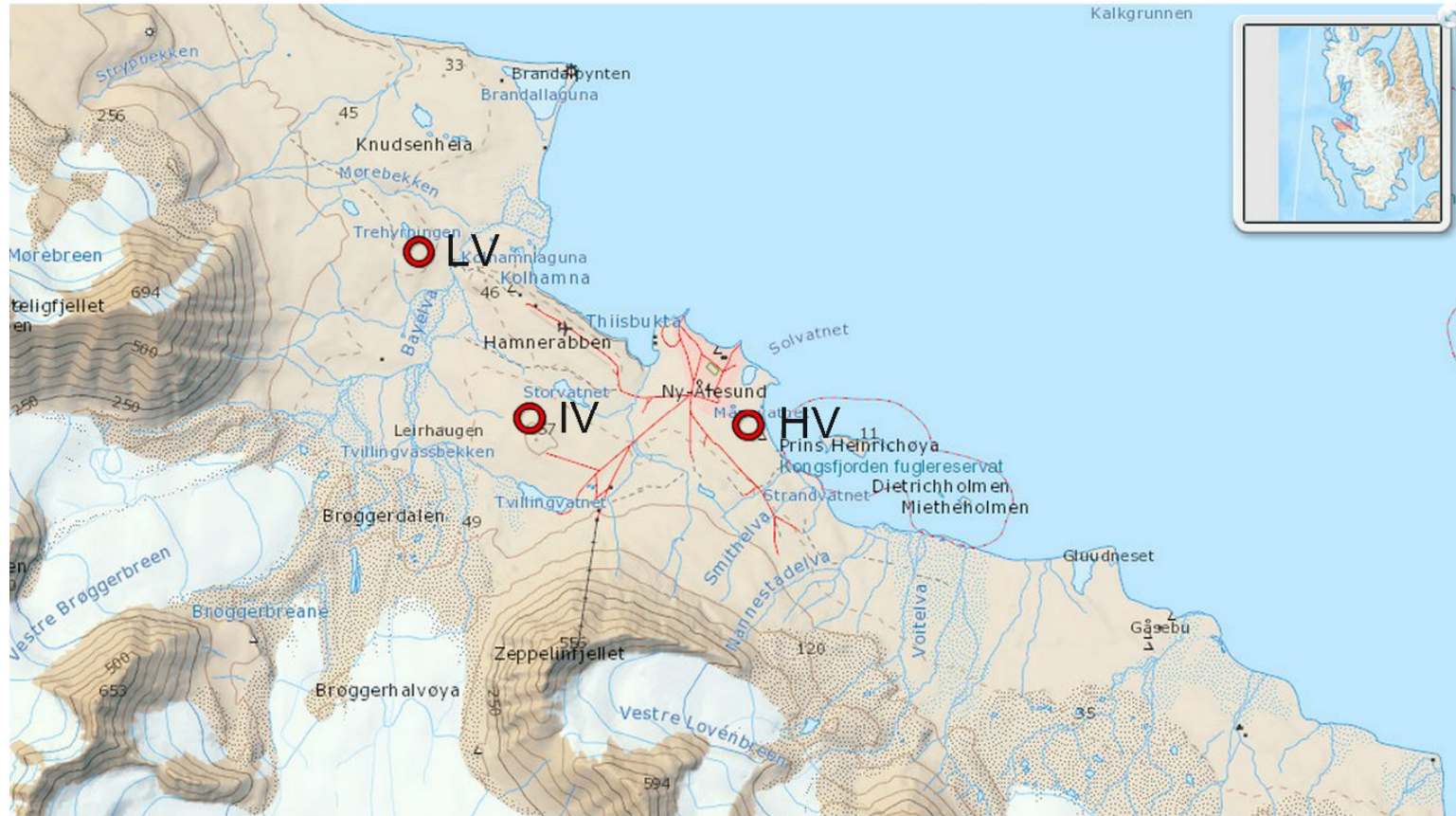
Fig. 1 Map of Svalbard and location of the each tundra sites: Lowest vegetation covered tundra (LV), Intermediated vegetation covered tundra (IV), Highest vegetation covered tundra (HV) (Modified after http:// toposvalbard.npolar.no/).

2.2.2 Sampling method.

Sampling was carried out on one site within each of the three types of tundra defined by vegetation cover. At each site, a range of sample area was 70m X 70m and within each sample area, five small quadrats (1m x 1m) at least 20m apart were chosen. After gently removing the surface moss and rocks, the top 5cm of soil underneath was collected from the four corners and center of each meter quadrat. The top 5cm soil from all these 5 points of the quadrat was collected into a single sampling bag and gently mixed before transporting it to Dasan facility for further processing. For characterizing the vegetation type, plant cover was recorded on each 1X1m quadrat by photography, before soil sampling took place.

2.2.3 Soil Metazoan DNA extraction.

At the Dasan Station, a 100g portion of each soil sample was processed to extract soil metazoan material, using a modified Baermann funnel technique (Thorne, 1961). To capture active soil Metazoa, the soil was gently sieved with 2mm sieve to remove pebbles and organic material then it was loaded placed into funnels. After 24-36 hours, Metazoa (mostly nematodes since these methods were originally designed for nematodes) were collected from the base of the funnel. Less active/dead metazoan components were captured by subjecting the same soil to sugar flotation (Jenkins, 1964).

2.2.4 DNA extraction, PCR and pyrosequencing of 18SrRNA gene.

The extracted organisms were concentrated into a small pellet by centrifugation (2,000 RPM for 30 seconds), and total DNA extracted using a MoBio PowerSoil DNA isolation kit according to the manufacturer's

instructions.

The DNA extracted separately from the Baerman Funnel and sugar flotation were later combined and used as a PCR template for amplification of a ~400bp diagnostic region, defined by primers NF1 (C. elegans numbering 1226-1250) and 18Sr2b (C.elegans numbering 1567-1588) towards the 3' end of the 18S rDNA (Porazinska et al., 2009). Purified amplified product was pyrosequenced using a 454 GS-FLX Titanium system (Roche).

2.2.5 Sequence processing.

Generated sequences were processed following Mothur's 454 SOP (Schloss et al., 2009) utilizing SILVA 115 for both alignment and taxonomic identification. Sequences were denoised including steps of trimming to remove primer and barcode sequences using an oligos file in addition of deleting shorter sequences (<150 nt) with homo-polymers

longer than 8 nt. Then, the sequences were aligned against the SILVA 115

eukaryotic database followed by screening step. Next, among those

remaining sequences, erroneous sequences were removed using pre-

cluster command followed by chimera detection by UCHIME (Edgar et al.,

2011). Taxonomic classification of Metazoa was performed against the

SILVA 115 eukaryotic database at a Bayesian cut-off 50%. Singletons

were removed using split.abund command in Mothur. Metazoans were

grouped into Operational taxonomic units (OTUs) at ≥99% similarity on a

subsample of (Metazoa=856, Nematoda=432), for calculating richness,

diversity and community compositional indices and matrices to be used

later for the statistical analyses.

2.2.6 Statistical analysis.

I performed sub.sample command (http://www.mothur.org/wiki/Sub.sample)

in Mothur to standardize sequences per sample. For soil Metazoa, samples

were standardized 856 reads and 432 reads for nematode.

Operational taxonomic unit (OTUs) (at 99% similarity) and diversity indices such as Shannon and OTU richness were processed with Mothur platform (Schloss et al., 2009). α−diversity of different tundra, using OTU richness diversity indices, was pairwise compared using ANOVA test in R. For β−diversity I used two methods, community β−diversity (Anderson et al., 2011) and true β−diversity (Koleff et al., 2003). Community β−diversity, community dissimilarity−based metric based on OTUs, is measured in a sense of variation in community using betadisper function in R. It shows the average distance from group centroid to each sampling point. True β−diversity which compares the total OTU richness to the average OTU richness was calculated by following equation: $S/\bar{\alpha} = (a + b + c)/[(2a + b + c)/2]$; S: total number of OTUs of two samples, $\bar{\alpha}$: average number of OTUs of two sample, a: shared OTUs of two samples, b: OTU only found in sample1, c: OTU only found in sample2. To compare β−diversity for both methods between different tundra, I performed

pairwise comparison using Post hoc Tukey tests.

For analyzing community similarity, I calculated unweighted UniFrac accompanied with ANOSIM test, which measures sequence difference between samples based on phylogenetic information. I used non-metric multidimensional scaling plots (NMDS) to plot metazoan/ nematode community structure according to the unweighted Unifrac with ANOSIM statistical test using primer6 (Clarke and Gorley, 2006) to visualize community composition. Percentage Relative abundance of different metazoan classes and nematode feeding groups of three tundra were calculated based on number of reads. In case of nematode feeding group, I chose top 15 abundant nematode family. For the normal distributed variables I used Kruskal-Wallis test, but for the variables not normally distributed I used ANOVA test for comparison.

Environmental variables (i.e. pH, TOC, P_2O_5, salinity, TN and C/N) per quadrat were used to assess the relationship of metazoan/nematode α-diversity with each environmental variable. I estimated dissimilarity in

environmental variables using Euclidean distance. To investigate the relationship between diversity of Metazoa (or nematode) and environmental variables, multiple regression was performed. Before that, I confirmed that each variables were not correlated each other, removing redundant variables using Varclus test which uses the square of Spearman's rank correlation (a non-parametric correlation) in R, Hmisc package. Then I used linear models for normal data, or generalized linear models for not normal data. As a diversity indices, OTU richness, Shannon, Inverse Simpson, Ace, Chao index were used. To evaluate if community composition (i.e. Unifrac) was structured in relation to any of the environmental variables measured I used the envfit function in package Vegan in R version 3.0.1.

2.2.7 Soil analysis.

Soil pH, total nitrogen, available phosphorus, Salinity and total carbon were

measured based on the standard protocol of SSSA (Soil Science Society of America) at National Instrumentation Center for Environmental Management (NICEM, South Korea).

2.3 Results

2.3.1 General findings.

Overall, 499 different Metazoa OTUs and 314 Nematoda OTUs were found across all three tundra types. Out of 499 OTUs in metazoa16.03% of OTUs were shared between HV and IV, 15.83% between HV and LV, and 15.63% between IV and LV. Only 11.62% of all OTUs were shared across all vegetation types.

For nematodes, total 135 OTUs were found in HV tundra, 156 OTUs in IV and 157 OTUs in LV tundra. Out of 314 Nematoda OTUs, 20.38% of OTUs were shared between HV and IV, 17.83% between HV and LV, and 17.83% between IV and LV. 13.38% of OTUs was shared across all three

tundra types.

We chose most abundant (in terms of number of OTUs) 22 families

of metazoan OTUs from three tundra sites (Table 1). The total number of

OTUs showed that the most dominant phylum at all sites overall was

Nematoda (family Qudsianematoae) but Tardigrada (family Hypsibiidae)

was the most dominant phylum at IV and LV. In case of Arthropoda,

Collembola was found than in greater abundance than Arachnida overall,

except at IV tundra.

Phylum	Class	Family	Feeding group	HV	IV	LV	Total # of OTUs
Nematoda	Enoplea	Qudsianematidae	Omnivorous	572	466	531	1569
Tardigrada	-	Hypsibiidae		125	693	629	1447
Arthropoda	Collembola	-		111	430	628	1169
Nematoda	Chromadorea	Dolichodoridae	Plant feeding	372	203	461	1036
Arthropoda	Arachnida	-		54	670	128	852
Nematoda	Chromadorea	Tylenchidae	Plant feeding	313	170	301	784
Nematoda	Chromadorea	Plectidae	Bacterial feeding	157	282	213	652
Nematoda	Enoplea	Mermithidae	Insect parasite	382	50	112	544
Rotifera	-	Philodinidae		214	165	84	463
Nematoda	Chromadorea	Cephalobidae	Bacterial feeding	24	203	133	360
Nematoda	Enoplea	Mononchidae	Animal predation	47	73	239	359
Nematoda	Chromadorea	Teratocephalidae	Bacterial feeding	223	48	78	349
Nematoda	Chromadorea	Chromadoridae	Bacterial feeding	152	86	60	298
Nematoda	Enoplea	Prismatolaimidae	Bacterial feeding	85	69	68	222
Nematoda	Enoplea	Nygolaimidae	Animal predation	11	0	166	177
Nematoda	Enoplea	Bastianiidae	Bacterial feeding	92	6	66	164
Nematoda	Chromadorea	Monhysteridae	Bacterial feeding	70	31	46	147
Nematoda	Enoplea	Tripylidae	Animal predation	130	2	0	132
Nematoda	Enoplea	Dorylaimidae	Omnivorous	15	54	7	76
Nematoda	Chromadorea	Aphelenchida	Fungal feeding	6	12	54	72
Annelida	-	Family Incertae sedis		52	6	3	61
Platyhelminthes	Turbellaria	Rhabdocoela		37	0	0	37

Table 1. Most abundant 22 identified metazoan OTUs (to family level) overall from the three tundra sites.

2.3.2 α − and β −diversity in the main tundra types of Svalbard.

To compare α −diversity, I analyzed pairwise comparison using OTU richness for both Nematoda and other Metazoa from different tundra types. Even though each tundra type has different vegetation cover and soil properties (ANOSIM: IV−LV: R=0.744, *P <0.05; LV−HV: R=0.88, *P <0.05), neither Metazoa nor Nematoda showed significant differences in α −diversity between the three tundra types (*P <0.05).

For Metazoa, Post hoc Tukey tests revealed that true β −diversity of LV tundra and of IV each harbored significantly higher true β −diversity than HV (*P <0.05). This trend was found for both all Metazoa combined (Annelida, Arthropoda, Craniata, Gastrotricha, Nematoda, Platyhelminthes, Rotifera and Tardigrada) and Nematoda alone (*P <0.05) (Fig. 2). I also analyzed distance to centroid as a measure of community β −diversity (Anderson et al., 2011), finding that the metazoan community from IV tundra and HV tundra are significantly different (*P <0.05), with IV having

higher β-diversity than HV tundra (Supplementary Fig. S1). Nematoda

alone also followed the same pattern (*P <0.05) (Fig. 2).
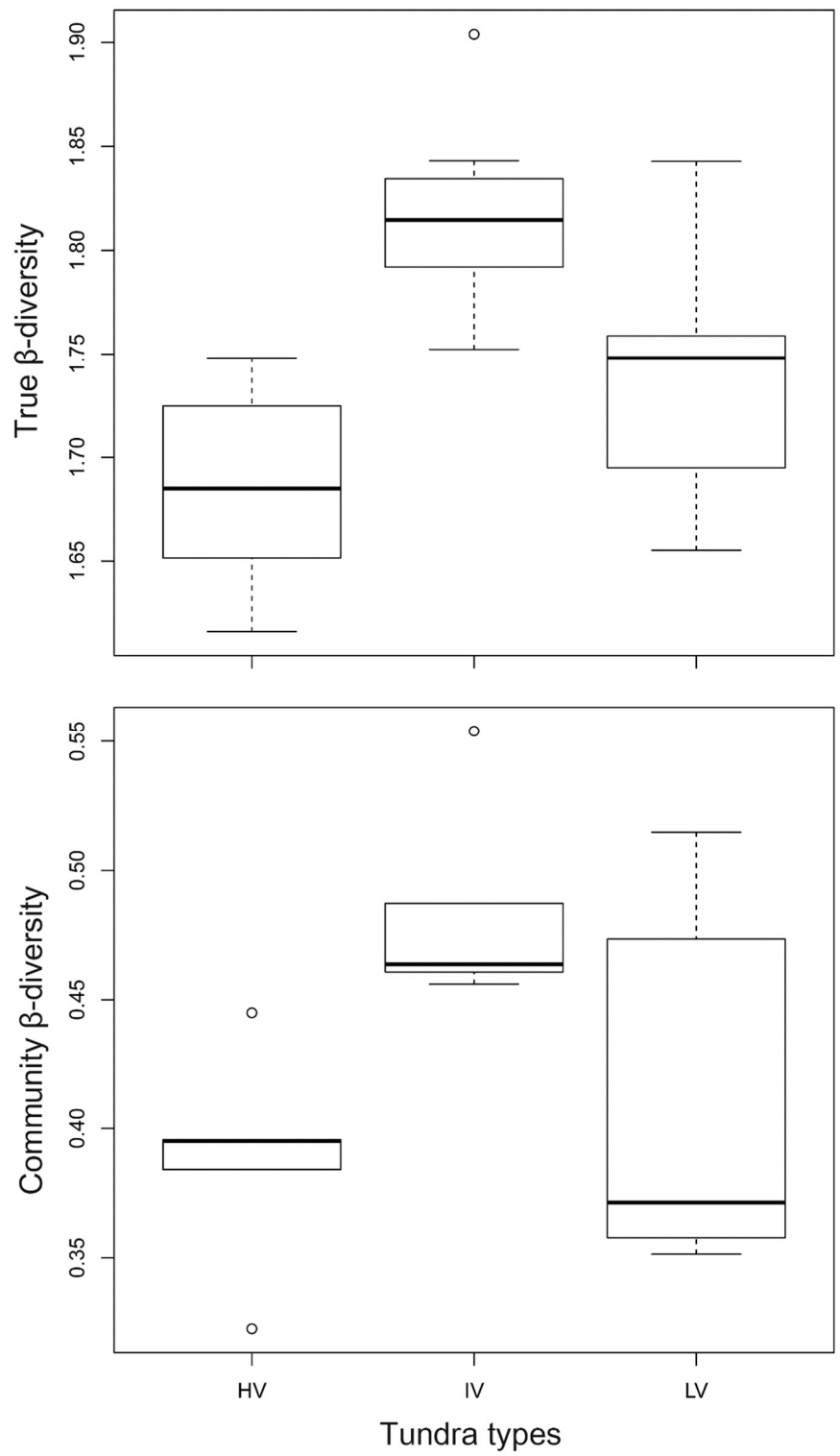
Fig. 2 β diversity of nematode in three different tundra shows that IV

tundra harbors higher β diversity than HV tundra (P < 0.05).

2.3.3 Difference in Metazoa community composition among tundra types.

In terms of the total Metazoa community composition, ANOSIM test and NMDS plots using unweighted UniFrac dissimilarity showed that there was a significant statistical difference (R=0.264, *P <0.05) between the HV and LV sites (Supplementary Fig. S2). A similar pattern (R=0.304, *P <0.05) was also observed for Nematoda only (Fig. 3).

In the case of relative abundance based on number of reads, pairwise comparison showed that there were no significant differences in terms of metazoan phyla or different feeding groups within nematodes between different tundra (*P <0.05) (Fig. 4; Table 2). Qudsianematidae, which belongs to the omnivorous feeding group, was the most dominant family of nematodes. Of all nematodes, the omnivorous feeding group was the third most abundant with 22.08% of all reads within the nematodes and 13.47% of all reads in Metazoan combined.
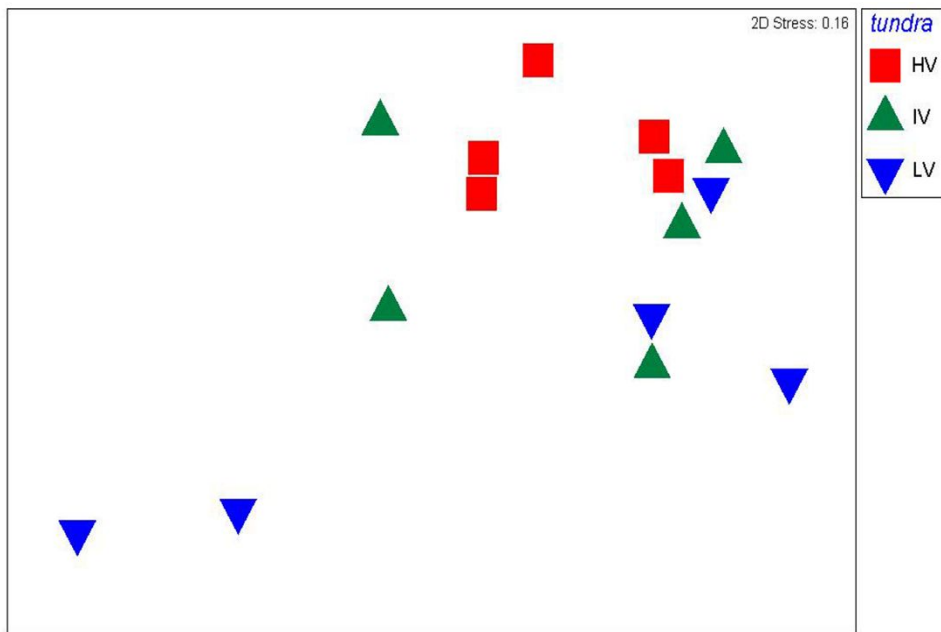
Fig. 3 NMDS based on UniFrac distance of nematode in three different tundra shows that HV and LV tundra harbors distinct nematode community.
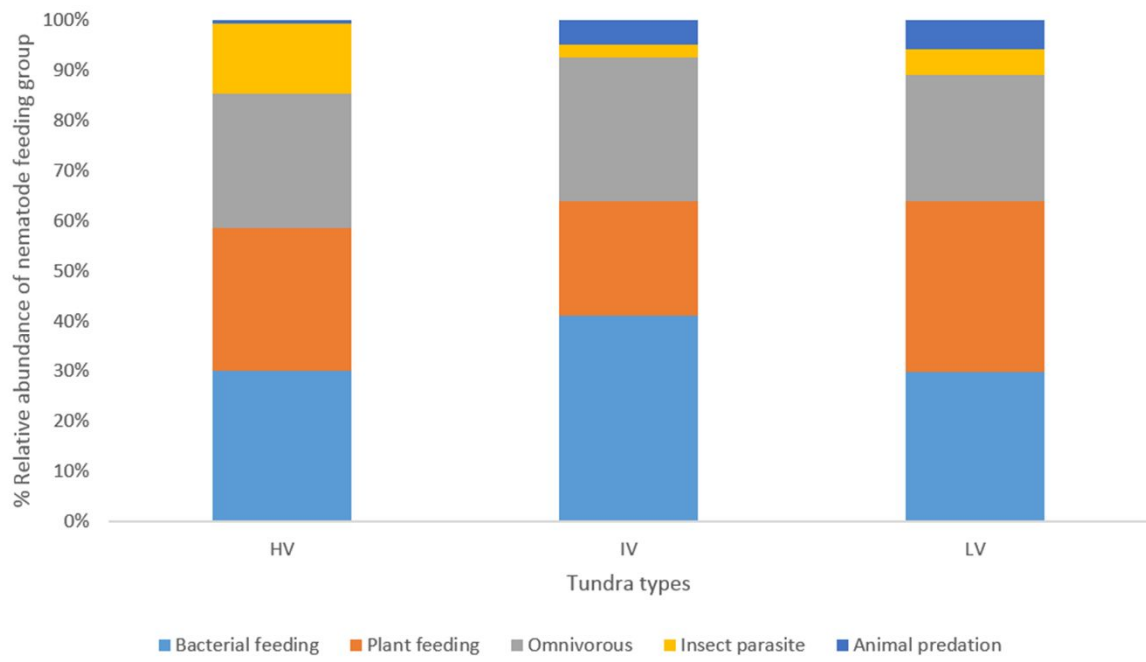
Fig. 4 % Relative abundance of different metazoan classes.

Categorization		% Relative abundance of different feeding group		
Feeding group	Family	HV	IV	LV
Bacterial feeding	Bastianiidae Cephalobidae Chromadoridae Monhysteridae Plectidae Prismatolaimidae Teratocephalidae	30.0405954	41.20505	29.76942
Plant feeding	Criconematidae Dolichodoridae Tylenchidae	28.61009086	22.78912	34.32222
Omnivorous	Dorylaimidae Qudsianematidae	26.79296346	28.58763	24.98164
Insect parasite	Mermithidae	13.95708486	2.575316	5.066823
Animal predation	Mononchidae Nygolaimidae	0.599265417	4.84289	5.859891

% Relative abundance was calculated based on number of reads.

Table 2 % Relative abundance of top 15 dominant nematode families of different feeding groups and their feeding

preference.

2.3.4 Soil parameters predicting Metazoa diversity and relative abundance within the Svalbard tundra.

ANOSIM result and NMDS based on Euclidean distance revealed that some tundra type appeared to have a distinct soil environment (LV-HV: R=0.88, *P <0.05, LV-IV: R=0.744, *P <0.05) (Supplementary Fig. S3).

The Envfit function in R showed that of the soil parameters measured, only salinity was marginally significant as a structuring factor in the Metazoa community composition (P=0.057). In the case of Nematoda, none of the soil parameters was found significant as a structuring factor of community composition.

To assess if there was correlation between the soil parameters measured, I used the Varclus test. I did not find a strong correlation between any pair of the environmental variables (TN- P_2O_5: $\rho 2=0.5$, TOC-CN: $\rho 2=0.45$, pH-Salinity: $\rho 2=0$). Therefore, all six environmental variables (TN, P_2O_5, TOC, CN, pH and Salinity) were used

for multiple regression. I found a TOC, C/N ratio and P_2O_5 are correlated only with metazoan Shannon index (Table. 3) reflecting that those environment variables positively affect metazoan α diversity. In case of Nematoda, no environmental factors were correlated with α diversity.

	Df	Deviance	AIC	F value	Pr(F)
TOC	1	5.0353	33.414	4.4602	0.06085
CN	1	5.2217	33.923	4.9952	0.04942 *
P_2O_5	1	5.5182	34.696	5.8467	0.03619 *

P ≤0 '***' 0.001 '**' 0.01 '*' 0.05. The analysis was performed with a Goodness of fit of the linear models

Table 3 Regression analysis of metazoan Shannon index in relation to soil properties.

2.4 Discussion

2.4.1 Nematode 'species' diversity detected by this metagenetic study is much higher than is detected by classical morphological studies.

This study found 314 OTUs of nematodes (proxy 'species' roughly corresponding to the taxonomic distances between species, at 99% sequence similarity) and 499 OTUs which belong to all Metazoa (including nematodes) across our tundra sampling sites in Svalbard. There appear to be many more species of Nematoda in our samples than previously found in other studies on Svalbard. Eight nematode genera were identified morphologically by Klekowski & Opalinski (1986) and 113 nematode species − including both soil and shallow freshwater habitats − identified by Coulson (2013). Comparison of our results with those by Klekowski & Opalinski showed the presence of particular species from three of the Nematoda genera they found, and members of the same families of the other five families found in their study. This overlap bolsters the

interpretation that the metagenetic approach offers an accurate, but more comprehensive, view of the nematode community. In addition, our study was able to find at least 14 families that the previous morphology-based studies on Svalbard had not identified. Although there was a certain amount of ambiguity in taxonomic level, this approach greatly facilitates the ecological study of nematodes in arctic ecosystems, compared to the traditional method. The metagenetic technique used here has also opened up the study of several additional groups of small terrestrial Metazoa on which very little or no work had been done on Svalbard due to the practical difficulties of studying them.

2.4.2 Differences in Nematoda α and β diversity between the different main tundra types of Svalbard.

A priori, greater plant biomass and (presumably) productivity in the HV tundra might be expected to provide for greater diversity of Metazoa by

providing enough material — both plant and microbial — to support Metazoa in diverse niches. However, I found no overall difference in Metazoa/Nematoda α diversity between the three tundra types I sampled.

When soil environmental variables were considered, TOC, C/N, P_2O_5 all showed positive correlation with Metazoa Shannon α diversity; P_2O_5 showed the most strong correlation followed by C/N and TOC however, only three of them together showed significant result. These results are in broad agreement with other studies in Antarctica and New Zealand (Barrett et al., 2008; Wall and Virginia, 1999; Yeates, 1977) which found a positive correlation between the amount of organic carbon and Metazoa diversity, especially Nematoda diversity. However, in our study the correlation was only for Metazoa not Nematoda.

While the three different tundra types are distinguishable by their percentage vegetation cover this appeared to affect neither overall Metazoa nor Nematoda α −diversity, whilst the predictions of Hooper et al. 2000 mentioned that plant species diversity enables more diverse soil

44

environment by secreting different root exudate. Our study showed that the less plant-species-rich, IV tundra and LV tundra, had higher metazoan/nematode β-diversity than the HV tundra, in terms of both community β-diversity (Anderson, Crist et al. 2011) and "true" β-diversity (Koleff, Gaston et al. 2003). Other studies (Knops et al., 2001; Porazinska et al., 2003; Wardle et al., 1997; Wardle and Nicholson, 1996) also found that plant species identity rather than plant species richness was more important in delimiting the diversity of soil Nematoda.

The apparent lack of importance of plant diversity variation for the soil Metazoa community might be explained by the fact that the Arctic ecosystem harbors relatively low plant diversity compared to other biomes (Jónsdóttir, 2005) and thus may exert insufficient diversifying effects on soil properties. It appears, then, that in the high arctic tundra, the soil Metazoa community composition is controlled mainly by soil properties rather than % plant cover.

It is generally held that in extreme environments such as desert or

polar ecosystems, species often show high spatial heterogeneity due to difficulty of accessing limited resources (Aguiar and Sala, 1999; Burke et al., 1999; Hoschitz and Kaufmann, 2004; Wall and Virginia, 1999). The fact that HV tundra showed lower β diversity than IV and LV tundra is indeed consistent with this broader ecological pattern, since local variation in surface cover is low in HV. In IV and LV tundra, I observed more many more bare patches (>30%), polygons (>10%), rocks and small pebbles (>20%). On the other hand, HV tundra was mostly covered with mosses with fewer bare patches (>60%) compared to IV and LV.

The mosaic nature of the IV and LV tundra might promote greater patchiness in nutrient supply as part of this environmental variation, as has been noted for other ecosystem types (Stafford Smith and Pickup, 1990). Nutrients such as N are often accumulated in the same spot as organic matter from plant litter is stacked. Also, seeds tend to be spread near those spots (Aguiar and Sala 1999).

Essentially the differences in β diversity of small Metazoa may be

explained in terms of the more pronounced landscape mosaic of bare and open areas within the LV tundra and IV tundra providing distinct sets of microenvironments, each with a distinct community of Metazoa. Combined with this, dispersal lag and population drift between the different patches might also contribute to variation in community composition from one local site to another.

2.4.3 Difference in nematode and total soil metazoan community composition between the different main tundra types of Svalbard.

I was interested in understanding whether there is evidence for spatial niche/habitat differentiation within the Svalbard tundra, or whether soil Metazoa in contrast seem to be generalized without strong local habitat preferences.

When the three tundra types were plotted on an NMDS based on their phylogenetic distance, HV and LV tundra showed distinct sets of lineages

of Nematoda and Metazoa from one another. In the case of Arthropoda, IV and LV tundra showed a distinct community composition from one another. Overall, these results suggest that the perceived 'tundra type' based on the percentage vegetation cover is a strong predictor of structuring of the total metazoan assemblages. There must be a significant degree of niche differentiation and specialization according to local environmental conditions in this landscape. This is despite the fact that the high arctic tundra is an 'extreme' environment, close to the limits of land surface ecosystems – species can still survive to some extent as 'specialists' in a subset of the conditions existing locally.

Generally, the IV tundra appears to be an ecotone between HV and LV. Samples belonging to the different tundra types harbor relatively distinct communities based on phylogenetic information. From this I may infer that either the fact that although metazoans (including Nematoda) are mobile, with their small body sizes their dispersal rate is somewhat confined to the local tundra type, without sufficient dispersal over the

several hundred meters distance between our tundra types to eliminate any spatial patterns or despite they can still disperse, each tundra provides adequate conditions for one's community.

ANOSIM and NMDS using Euclidean distance showed that there was a significant difference in terms of soil properties between HV and LV tundra. When Metazoan community composition was plotted using environmental variables as vectors across all the tundra samples combined, only salinity emerged as a structuring factor of metazoan community. Generally, however soil salinity was not high in any of our samples, and known halophytes were not present in any of our quadrat samples. Soil salinity here does not appear to directly reflect intensity of windblown salt spray input, since HV tundra had lower salinity. It is possible that the higher salinity of the LV tundra reflects more the generally drier soil conditions, against a background of salt input by local chemical weathering or small quantities of windblown salt. Similarly, various studies in Antarctica have found that nematode distribution is affected by soil salinity

49

(Courtright et al., 2001; Nkem et al., 2005).

Another indicator of community composition is relative abundance of reads, as a possible indicator of relative biomass abundance. When the total number of reads for all Metazoa was plotted by phyla, and within Nematoda alone, there was no statistical difference in abundance of major taxa between tundra types. From the combined samples overall, I chose the 15 most abundant families of Nematoda, and categorized them by functional diversity as previously defined by Yeates et al., (1993). Bacterial feeding nematode groups were dominant, followed by plant feeding and the least abundant was insect parasites. Family Qudsianematidae, which belongs to the omnivorous feeding group as described, was the most abundant family across the site. Although family Qudsianematidae is distributed in various regions from arctic to the Antarctic (Andrassy, 1998; Kito et al., 1996; Vinciguerra and Orselli, 1998) our result showed that among different species under family Qudsianematidae, Eudorylaimuscarteri constituted around 74% of the

family in our samples. Members of Eudorylaimus sp. are known to inhabit

various regions including Antarctic but most of them (70%) are especially

restricted to Palearctic (Andrássy, 1986).

CHAPTER3. MICROSITE DIFFERENTIATION IN SOIL METAZOAN COMMUNITIES WITHIN THE SVALBARD HIGH ARCTIC TUNDRA.

3.1 Introduction

One of the greatest challenges in understanding polar ecosystems is to determine how the patterns of diversity and community composition of organisms adjust themselves to local microclimates, and differences in the availability of resources.

In general, polar ecosystem is considered as optimal place to study the ecology/ biology of soil communities predominantly because these systems are so much simpler, less complex, and thus predicting what factors structure the soil communities should be a much easier task than in other parts of the world.

The few previous studies of small soil animals in tundra or polar desert, have focused predominantly on the nematode community composition and structure with other metazoan phyla not included. Polar nematode studies

from the Dry Valleys of Antarctica revealed a low overall diversity of

nematodes (often 1－3 species in each microhabitat), but quite distinct

communities adapted to particular substrates and microclimates, often

separated by just a few meters (Kennedy, 1993; Powers et al., 1995;

Treonis et al., 1999; Porazinska et al., 2002; Freckman and Virginia, 1997).

In the polar semi－desert of Devon Island (Canada) distribution of

nematodes also depended on microsite (Cockell et al., 2001). 'Micro－

oases' of greater plant cover were associated with higher nutrient

concentrations, and with high populations of soil bacteria and fungi, had a

greater abundance and diversity of nematodes. In all of these studies,

while nematode diversity was restricted to only a few species, it was

greater in warmer, moister and more nutrient－rich microsites. An

exception was the study by Mouratov et al. (2001) on King George Island

(adjacent to the Antarctic Peninsula) where most samples from the sparse

tundra/polar semi desert yielded no more than 3 or 4 nematode species,

but the abundance and diversity were often lowest in the dampest

microsites - perhaps because of the generally too moist soil conditions resulting in waterlogged low-oxygen and low nutrient peaty soil environments..

Here, this study investigated soil Metazoa in high arctic soils of Svalbard (Norway) by using metagenetics (metabarcoding), a taxonomically inclusive approach that allows for a more complete picture of the diversity and composition of the soil metazoan community.

The study was structured around investigating the following questions: Are soil metazoan communities in different microsites (amongst plant roots, under moss clumps, on open cyanobacterial mat areas, and polygon) distinct from one another and thus indicating niche differentiation among different environments? If indeed they are distinct, what soil parameters best predict such distinct soil metazoan communities within the Svalbard tundra? Is the soil metazoan community specialized enough to be distinct between the root systems of different species of tundra plants?

3.2 Methodology.

3.2.1 Site description.

Fieldwork was conducted in Svalbard in late July 2013, corresponding to peak arctic summer temperatures, at sites within reach of the Dasan Base, at Koengsford, Svalbard.

In contrast to our earlier study (Park et al., submitted ms) which took square meter-scale samples on a regular grid pattern through three distinct tundra types, here I address a much finer scale of sampling – each sample being several centimeters across – to examine microsite effects. Samples were scattered equally across three main tundra types (high vegetation cover, intermediate vegetation cover, and low vegetation cover) at Kongsford. The three tundra types differed with respect to the amount of soil surface covered by plants,

The first sample area was located at latitude 78° 55′ 20.0″ N, longitude 11° 56′ 30.3″ E, within several hundred meters of the

coastline. This site had almost complete vegetation cover (high tundra type) of mostly bryophytes (particularly Dicranum, Polytrichum and Pohlia), dwarf willow (*Salix polaris* Wahlenb) and lichens (e.g. Cladonia), with small open areas of cyanobacterial mats. I took 4 rhizosphere samples from 4 individual plant specimens, 3 from underneath cyanobacterial mats and 4 from underneath Pohlia moss mats. Samples were taken as described below. The second sample area was a much more sparsely vegetated area (<60% vegetation cover, intermediate tundra type) with cyanobacterial mats and scattered angiosperm cushion plants, and mats and cushions of mosses (Polytrichum, Dicranum, Pohlia). It was located in latitude 78° 55′ 18.55″ N, longitude 11° 51′ 52.63″ E, covered mostly by few kinds of mosses and *Salix polaris* and frost/stone polygons were readily visible in this tundra type. Four rhizosphere of individual 4 plant species, 1 moss and 2 polygon samples were taken. The third site was known as semi−dry tundra and located in latitude 78° 55′ 58″ N, longitude 11° 49′ 22″ E. *Salix polaris* and *Carex fuliginosa* were the dominant vascular plants and the

overall vegetation cover was <50% (low tundra type). I took 3 rhizosphere

samples of individual 3 plant specimens, 3 cyanobacterial mat samples and

1 polygon sample.

3.2.2 Sampling method.

To capture very local microsite conditions, each microsite sample was

taken using a sterilized trowel from a surface area of approximately 10 cm

in diameter to a depth of 5 cm. Sampling was carried out on four different

kinds of microsites: plant rhizosphere (Fig 5 (a), (b)), under (<10cm)

cyanobacterial mats (without any vegetation cover (Fig 5 (c)), under

Pohlia moss mats (Fig 5 (d)) and stone polygon areas (Fig 5 (e)), each

with at least three replicates which is composite of multiple sample.

The microsite types were, in more detail:

1. Plant rhizosphere: I selected two of the most common cushion plant species found in all tundra sampling areas at Kongsford: *Carex parallela* and *Silene acaulis*. To collect rhizosphere soil, I gently removed the plant from the ground by levering it up with the blade of the trowel, and then collected soil from around the plant roots to a depth of 5cm. Eight rhizosphere soil samples were taken from the dicot cushion plant *Silene acaulis* and three samples were collected from the monocot cushion plant *Carex parallela*.

2. Under moss mats: I collected five samples from the thin soil layer (<2cm depth) attached to the underside of a Pohlia moss mat, concentrating on the B horizon material and avoiding excess organic matter being collected. Note that in this case I did not sample to 5 cm depth.

3. Stone polygons: Three soil samples were collected in the bare central area of stone polygons.

4. Cyanobacterial mat areases: I took five samples from under other localized flat areas soil without any vegetation cover. These bare areas covered with cyanobacteria were at least 30cm across, with the sample taken in the centre.

All collected soils were transferred immediately to a sampling bag and mixed gently before being moved to Dasan facility for the extraction of the soil Metazoa.
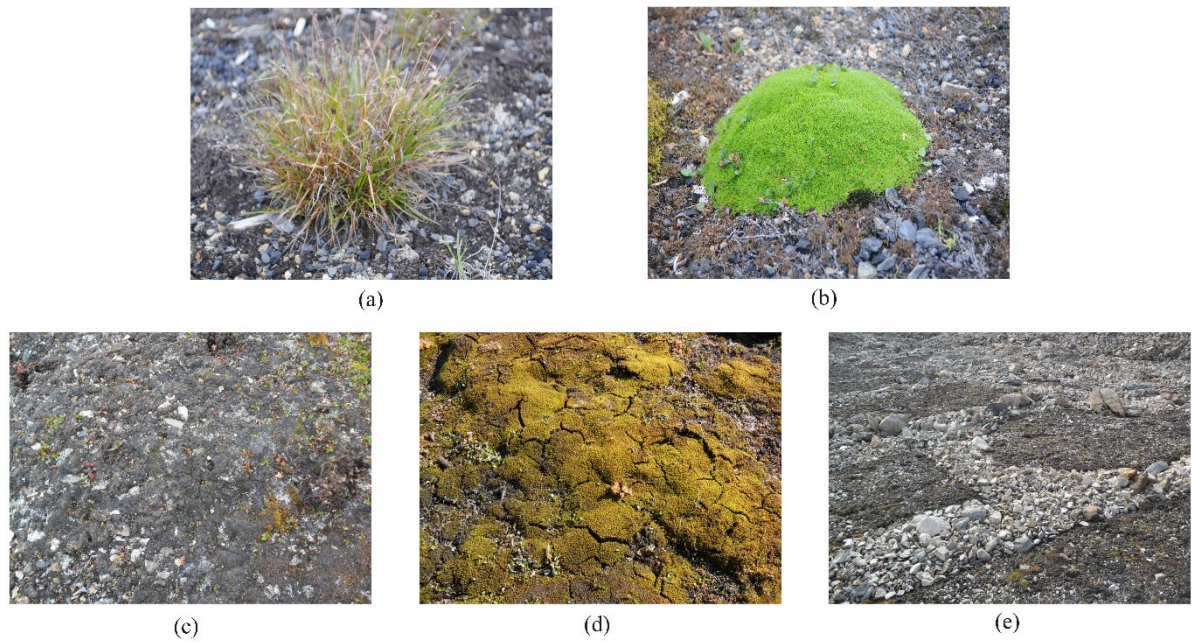
Fig. 5 Pictures of microsites. (a) Cushion plant (*Carex parallela*), (b) Cushion plant (*Silene acaulis*), (c) Cyanobacterial mat areas, (d) Moss mat areas, (e) Polygon.

3.2.3 Soil Metazoan DNA extraction.

100g wet weight of each soil sample was used for the DNA extraction at the Dasan station, using a modified Baermann funnel technique (Thorne, 1961) accompanied with sugar flotation (Jenkins, 1964). First, soil was loaded into a funnel after being gently sieved with a 2mm sieve to remove organic debris and small pebbles. Then, I added water into the funnel so that live metazoa could move down into the stem of the funnel. The fluid below the funnel containing metazoan bodies was collected after 24-36 hrs and centrifuged down to a pellet at 3000RPM. The soil remaining in the funnel was then subjected to sugar flotation for the less active/dead components (Jenkins, 1964). The decanted sugar solution containing suspended Metazoa was then spun down to a pellet at 3000RPM. (Fig.6)
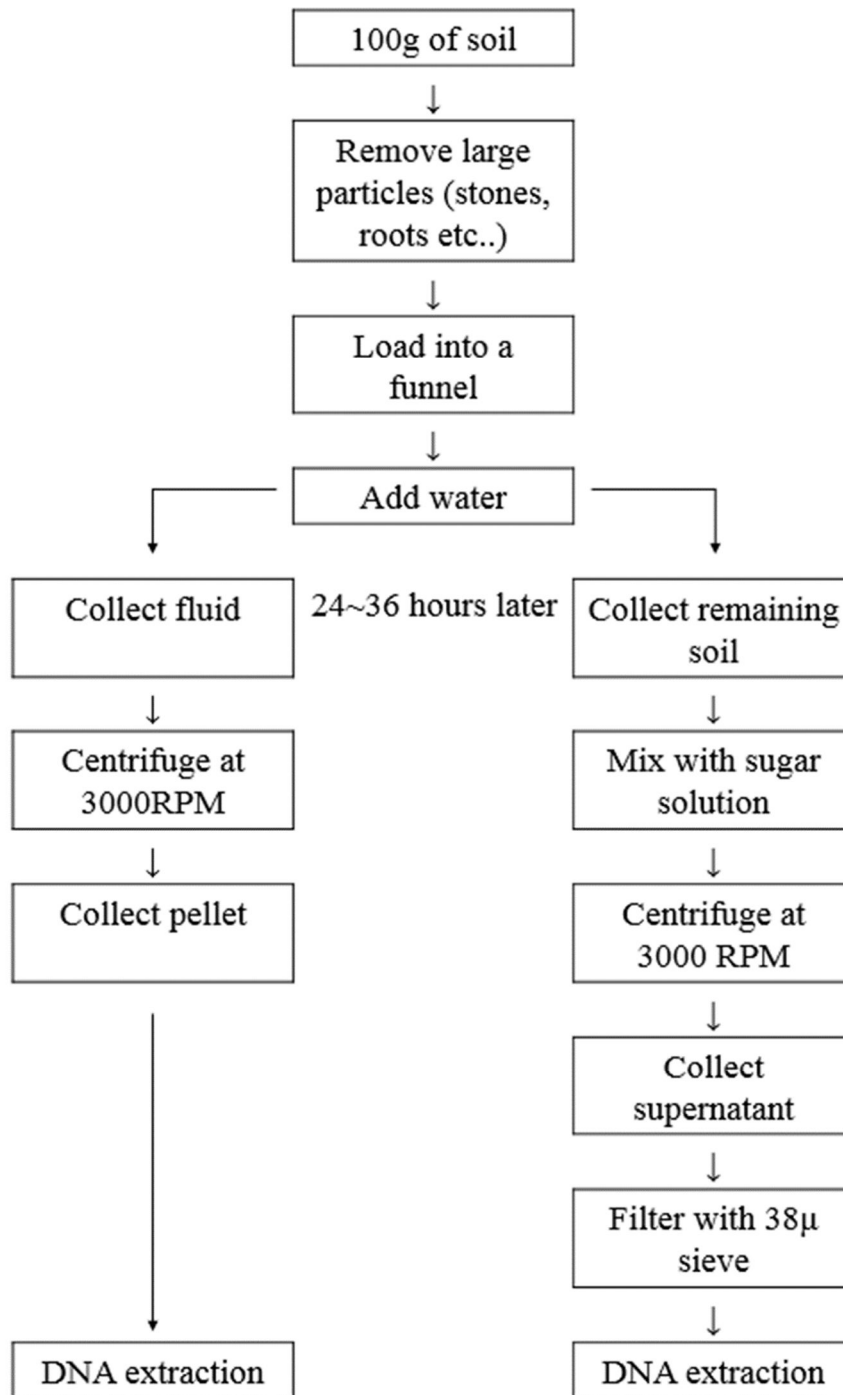
Fig. 6 Diagram of soil metazoan extraction procedure. The methodology combines Baermann Funnel and sugar flotation in order to sample the greatest range of taxa of soil Metazoa.

3.2.4 DNA extraction, PCR and pyrosequencing of 18SrRNA gene.

Total DNA was extracted from soil metazoan bodies at the Marine lab near Dasan Station using a MoBio PowerSoil DNA isolation kit according to the manufacturer's instructions.

Separate DNA extractions were done for the Baerman funnel and sugar flotation metazoan material, and stored at −20℃ before being transported back to SNU in Korea. There, the extracted DNA was combined in equal proportions and used as a PCR template for amplification of a ~400bp diagnostic region, defined by primers NF1 (5' −TCAG−CG−GGTGGTGCATGGCCGTTCTTAGTT−3') and 18Sr2b (5' −X−CCTACAAAGGGCAGGGACGTAAT−3') (Mullin et al., 2003) where X represents barcode varies from 6−8 base pairs. PCR products were purified using QIA quick PCR purification kit following manufacturer's instruction and pyrosequenced using a 454 GS−FLX Titanium system (Roche) sequenced on two 1/8 PicoTiterPlate run.

3.2.5 Sequence processing.

Sequences obtained from pyrosequencing were processed using Mothur (Scholoss et al., 2009) except for the chimera detection step. For sequence alignment and taxonomic identification, I used SILVA 115. Briefly, sequences less than 150 nt with homopolymers longer than 8nt and incorrect primer sequences or any ambiguous base calls of sequences were removed first. The remaining sequences were aligned against the SILVA 115 eukaryotic database. Next, I used UCHIME (Edgar et al., 2011) to detect chimeric sequences after the step of removing erroneous sequences by pre-cluster command. Taxonomic classification of each OTU (clustered at >99% sequence similarity) was generated against the SILVA 115 eukaryotic database at a Bayesian cut-off 50%. Before conducting any statistical analysis using community compositional indices, matrices and richness, I subsampled sequences from each sample to standardize the data using the sub.sample command (http://www.mothur.org/wiki/Sub.sample)

after the classification step, for Metazoa=856 and for Nematoda=432 ,which were the lowest number of reads across the samples .

3.2.6 Statistical analysis.

To visualize community similarity, I conducted a Non-metric Multi Dimensional Scaling (NMDS) based on Bray Curtis similarity matrix using Primer v6 (Clarke and Gorley, 2006) accompanied with ANOSIM statistical test, which measures sequence difference among samples from different microsites. I used pH, TOC, P_2O_5 and TN (see below) of each microsite as environmental co-variables to see if any of them played a role in structuring distinct nematode communities. For this, I used the envfit function in package Vegan in R version 3.0.1.

Classification by feeding guild was only possible for Phylum Nematoda, for which detailed information exists (Yeates, 1993). To see if the composition of the various nematode feeding groups differed among

microsites, I used percentage relative abundance of sequence reads, estimated from numbers of reads.

The most abundant 15 nematode families in our Svalbard samples were assigned to trophic groups (Yeates et al., 1993). Prior to statistical analyses, all the variables of relative abundance were checked for normality. Normally-distributed variables were analyzed using ANOVA test and variables not normally-distributed were analyzed using Kruskal-Wallis test. Multiple regression was performed to reveal whether any environmental variables correlated with diversity of rhizosphere nematodes or nematodes of cyanobacterial mat areas. Operational taxonomic unit (OTUs) (at 99% sequence similarity) and diversity indices such as inverse Simpson, Chao and ace indices were performed with the Mothur platform (Schloss et al., 2009).

For the soil properties, I first checked whether there were any redundant variables using the Varclus test which uses the square of Spearman's rank correlation (a non-parametric correlation) in R, Hmisc

package.

3.2.7 Soil analysis.

Soil from each microsite sample, in excess of that needed for the funnel extraction, was dried at the Dasan Base, and transported to Korea. Soil properties including pH, total nitrogen, total organic carbon and available phosphorus were measured based on the standard protocol of SSSA (Soil Science Society of America) at National Instrumentation Center for Environmental Management (NICEM, South Korea).

3.3 Results.

3.3.1 General findings.

A total of 123,249 quality eukaryotic sequences were classified at $\geq 99\%$ similarity level, distributed across all 25 microsite samples. Among those sequences, around 80.83% were classified into 648 Metazoa OTUs. Nematode OTUs made up 65.94% of all metazoan sequences and these were clustered into 420 nematode OTUs.

By microsite type, a total of 236 metazoan OTUs were found in cyanobacterial mat areas, 260 under moss cushions, 125 in polygons and 342 OTUs were found in rhizosphere samples. In case of nematodes alone, I found 154 OTUs in cyanobacterial mat areas, 156 under moss, 85 in polygons and 240 in rhizosphere samples.

3.3.2 Community composition of each microsite type.

NMDS based on Bray－Curtis and ANOSIM result revealed that the Metazoa community in rhizosphere samples differed from polygon samples (ANOSIM: R statistic=0.646, p<0.05), moss samples (ANOSIM: R statistic=0.288, p<0.05), and cyanobacterial mat areas (ANOSIM: R statistic=0.202, p<0.05). In addition, the metazoan community in polygon samples was distinct from that of cyanobacterial mat areas (ANOSIM: R statistic=0.519, p<0.05).

In case of the Nematoda community, rhizosphere samples differed from polygon samples (ANOSIM: R statistic=0.744, p<0.05), moss (ANOSIM: R statistic=0.418, p<0.05), and cyanobacterial mat area samples (ANOSIM: R statistic=0.303, p<0.05). Also cyanobacterial mat areas samples differed from polygon samples (ANOSIM: R statistic=0.549, p<0.05) and moss samples (ANOSIM: R statistic=0.384, p<0.05).

Additionally, environmental variables such as total nitrogen, available phosphorus and total carbon were indicated as major structuring factors of the nematode community composition (p<0.05) (Fig. 7). In case

of Metazoa, pH, total nitrogen, available phosphorus and total carbon were

the factors that delimits metazoan community of different microsites

(p<0.05) (Fig. 7).

NMDS of soil properties based on Euclidean distance and ANOSIM

test showed that soil properties including pH, TOC, TN and P_2O_5 were

significantly distinct between moss mats and cyanobacterial mat: R=0.532,

p<0.05, moss and *Silene acaulis*: R=0.582, p<0.05, polygon and *Silene*

acaulis: R=0.373, p<0.05 and there was marginally significant difference

between moss mat and polygon having R=0.6, p=0.054 (Fig. 8).

Nematoda was the most dominant phylum among soil Metazoa

(65.94 % of reads) followed by Arthropoda (13.67%), Tardigrada (9.53%),

Annelida (6.83%), Rotifera (2.76%), Gastrotricha (0.73), Platyhelminthes

(0.51%) and Craniata(0.02%) in all microsites. There was no significant

difference of % relative abundance of phyla/classes among microsites.

I chose the 15 most abundant (by sequencing reads) nematode

families, and classified them by their feeding preferences (Yeates et al.,

7 0

1993). Only percentage relative abundance of plant-feeding nematodes was higher in cyanobacterial mat areas than other microsites (Fig. 9).
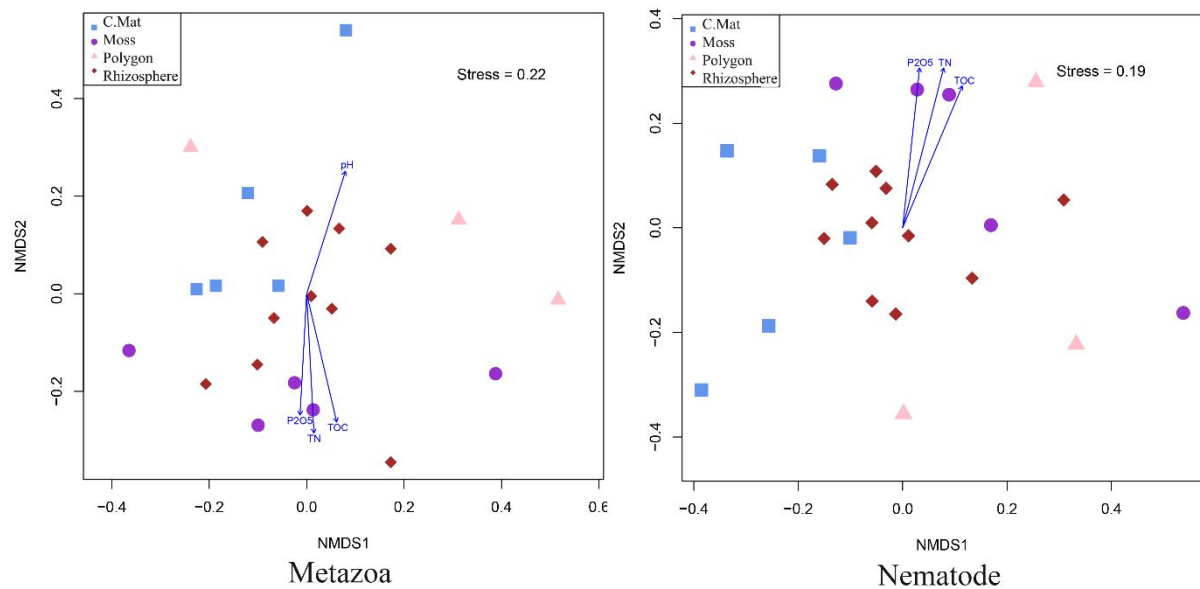
Fig. 7 Vector of environmental variables added to NMDS based on Bray-Curtis of total community of Metazoa (left) and Nematoda alone (right) of different microsites. Stress value measures the deviation of departure from monotonicity of the original n-dimensional space and distance in the reduced k-dimensional ordination space (McCune and Grace, 2002). C.Mat stands for cyanobacterial mat.
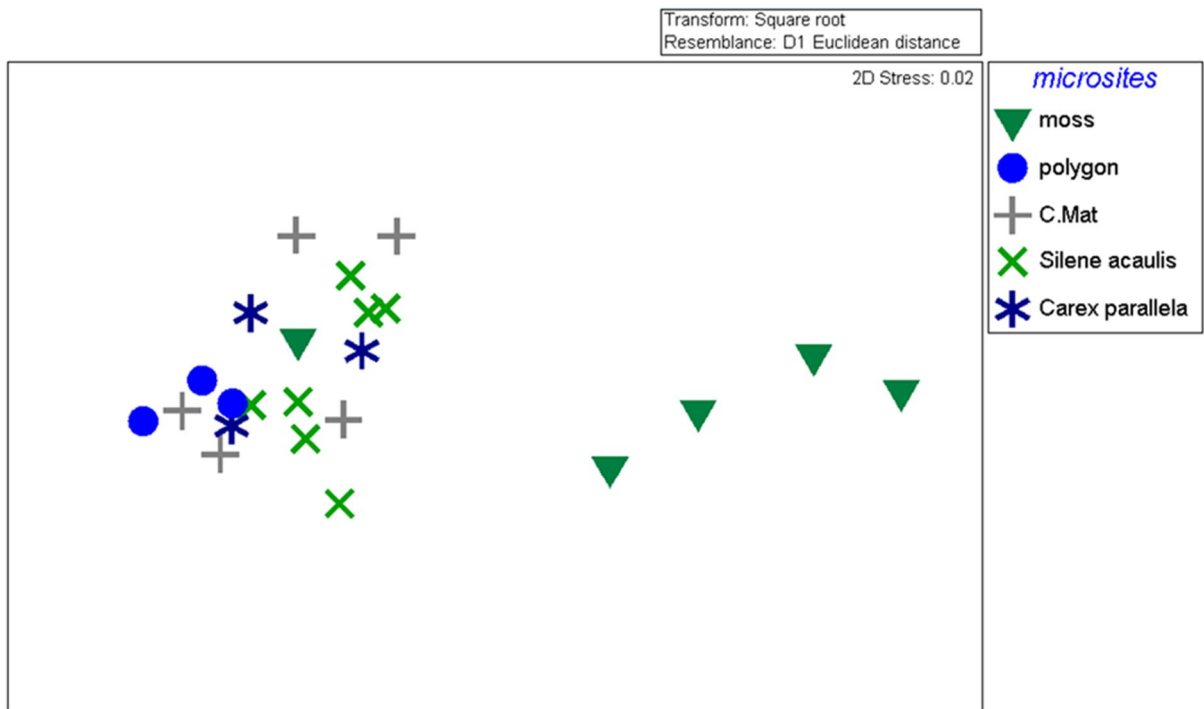
Fig. 8 NMDS of soil properties alone, based on Euclidean distance.
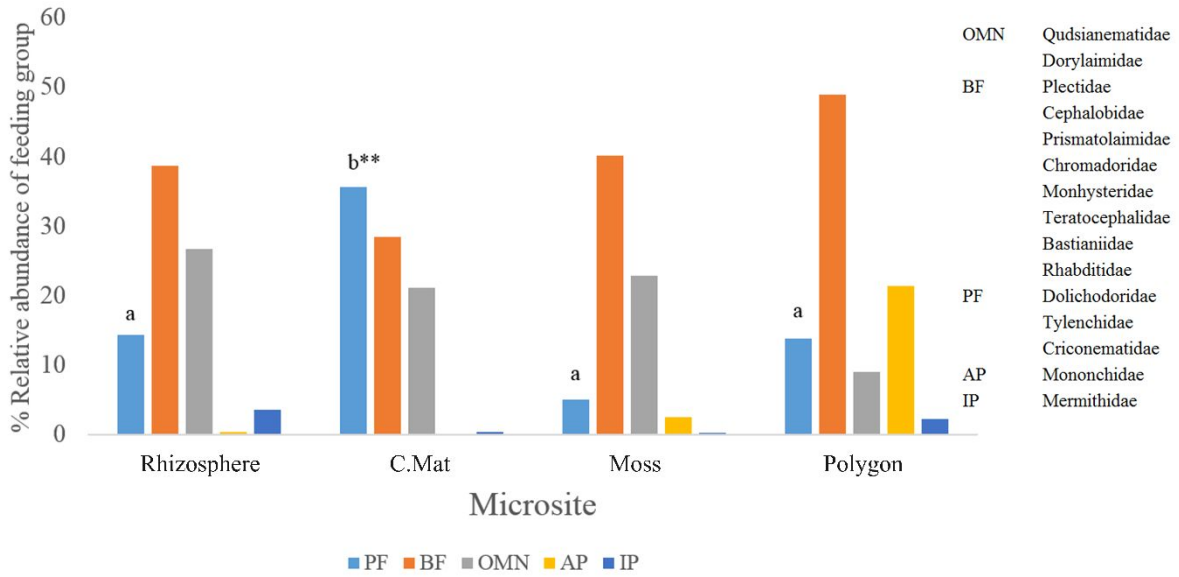
C.Mat: Cyanobacterial mat, PF: Plant feeding, BF: Bacterial feeding, OMN: Omnivore, AP: Animal parasite, IP: Insect parasite. Different letters represent significant difference between them.

Fig. 9 Putative percentage relative abundance (by reads) of nematode feeding groups in different microsites, based on numbers of reads falling into each category. C.Mat stands for cyanobacterial mat.

3.3.3 Rhizosphere metazoan community of two different plant species.

When rhizosphere samples from the two different cushion plant species (*Silene* and *Carex*) were compared, NMDS (based on Bray-Curtis) and ANOSIM results showed no significant difference ($p < 0.05$) in metazoan or nematode community. However, when total rhizosphere samples were compared with cyanobacterial mat areas, the two had a distinct community composition (Metazoa: ANOSIM: R statistic=0.202, $p < 0.05$, nematode: ANOSIM: R statistic=0.303, $p < 0.05$). When soil parameters were added, total organic carbon, total nitrogen and available phosphorus were the structuring factors of metazoan communities of rhizospheres and cyanobacterial mat areas (Fig. 10). On the other hand, only total organic carbon apparently impacted the distinct nematode community composition of rhizosphere and cyanobacterial mat areas samples ($p < 0.05$) (Fig. 10).

To understand whether any of the soil property variables were correlated with diversity of Metazoa in cyanobacterial mat and rhizosphere

microsites, a multiple regression analysis was performed. Since Varclus test indicated that pH, TOC, TN and P_2O_5 were independent from each other (pH$-$TN: $\rho 2=0.01$, pH$-$TOC: $\rho 2=0.08$, pH$- P_2O_5$: $\rho 2=0.21$, TN$-$TOC: $\rho 2=0.11$, TN$- P_2O_5$: $\rho 2=0.52$, TOC$- P_2O_5$: $\rho 2=0.12$), all four variables were used in the multiple regression test. Metazoan Shannon diversity was correlated with pH, total nitrogen and available phosphorus (Table 4). In addition, Simpson index was correlated with total nitrogen and inverse Simpson was correlated with pH. In the case of Nematoda OTUs of rhizosphere, inverse Simpson, Chao and ACE diversity indices correlated with pH. Chao and ACE were also correlated with total nitrogen and available phosphorus (Table 5). However, neither Metazoa nor Nematode diversity of the set of cyanobacterial mat areas samples show any significant relationship with any soil parameter at $p<0.05$.
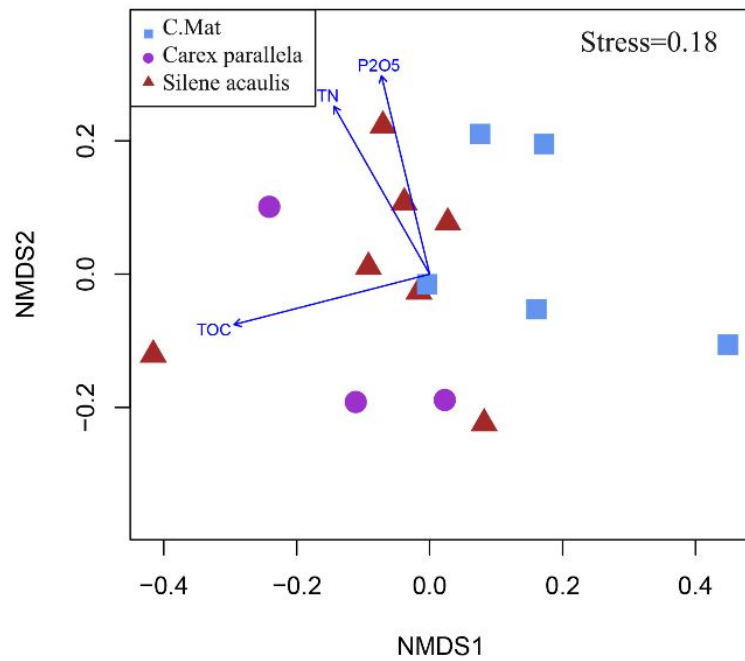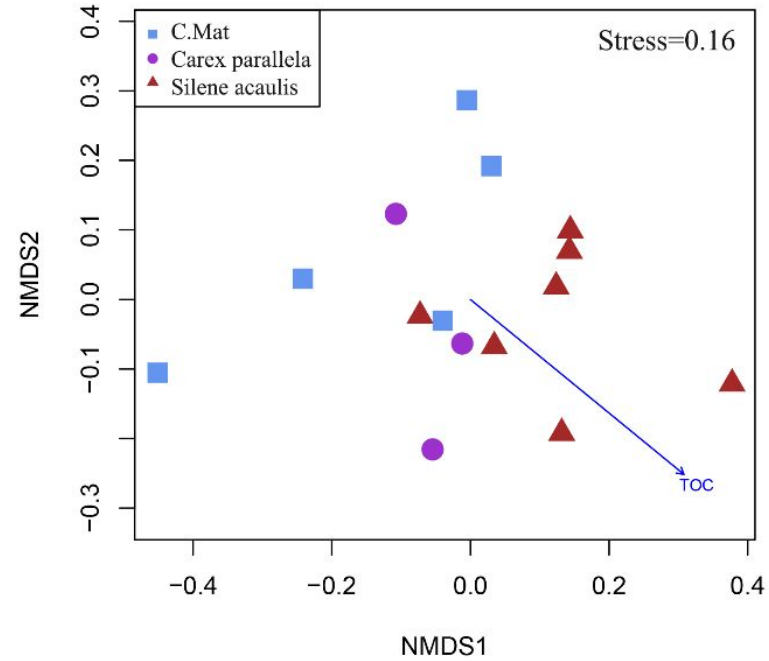
Fig.10 NMDS of Community composition of rhzosphere of 2 plant species (*Carex parallela, Silene acaulis*) and cyanobacterial mat (represented as C.Mat) with environmental variables included.

Table 4 Multiple regression of Metazoa diversity indices defined at 99% sequence similarity of 10 rhizosphere samples with soil properties.

		Linear multiple regression				
	Df	Sum of Sq	RSS	AIC	F value	Pr(F)
Shannon~pH	1	1.23609	1.7694	-11.319	13.9066	0.009746 **
Shannon~TN	1	1.2161	1.74941	-11.433	13.6817	0.010102 *
Shannon~P_2O_5	1	0.48773	1.02104	-16.818	5.4872	0.057649 .
Simpson~TN	1	0.051082	0.096416	-44.417	9.0144	0.01701 *
Inverse simpson~pH	1	165.81	329.1	36.938	8.1236	0.02147 *

Table 5 Multiple regression of nematode diversity indices defined at 99% sequence similarity of 10 rhizosphere samples with soil properties.

		Linear multiple regression				
	Df	Sum of Sq	RSS	AIC	F value	Pr(F)
Inverse simpson~pH	1	59.983	116.65	26.566	8.4681	0.01959 *
Chao~pH	1	5515.3	6840.3	71.28	24.9747	0.002459 **
Chao~TN	1	1947.9	3273	63.909	8.8208	0.024958 *
Chao~P_2O_5	1	3530.2	4855.2	67.852	15.9857	0.007133 **
		Generalized linear multiple regression				
	Df	Deviance	AIC	F value	Pr(F)	
ACE~pH	1	15988	110.15	13.3018	0.01074 *	
ACE~TN	1	10481	105.93	6.6526	0.04181 *	
ACE~P_2O_5	1	15489	109.83	12.6985	0.01188 *	

3.4 Discussion.

3.4.1 There is a distinct community composition of Metazoa between different microsites.

A comparison of the total Metazoa community defined at 99% sequence similarity across a range of tundra microsites (cushion plant rhizosphere, under moss mats, under cyanobacterial mat, and the central area of individual polygons) revealed compositionally distinct communities between different microsite types. Nematoda, when considered separately, also showed such community differentiation. It appears that specialized habitat conditions, definable by available Phosphorus (P_2O_5), total Nitrogen (TN) and total organic Carbon (TOC) concentration, might have structuring effects of the metazoan (including nematode) communities in this high arctic environment.

The rhizosphere microsite was most different from other microsites, presumably partly related to the more abundant resources of plant material

(living and dead), the nutrients such as C that this provides, moister soil

conditions, and the microbial communities living near the root surface.

Generally in arctic ecology, it has been suggested that the rhizosphere is

able to support a more complex and diverse food web, and also related to

water retention by the roots system in these areas (Jónsdóttir, 2005), and

our findings support this.

3.4.2 Metazoan community of the rhizosphere is not affected by the species identity of the host plant.

Cushion plants are known to maintain a warmer and more humid

microclimate in the soil underneath them, compared to the surrounding soil

(Arroyo et al., 2003; Cabrera et al., 1998; I Badano et al., 2006; Körner,

2003). Metazoan communities could be affected via the microclimate (e.g.

temperature) or soil chemistry (e.g. pH, organic matter) (Arroyo et al.,

2003). In choosing two common cushion plant species of Svalbard, *Silene*

acaulis and *Carex parallela*, I hypothesized that a specialized plant-

parasitic nematode community (and perhaps decomposers and predators as

well) might exist under each plant species as has been documented in

warmer parts of the world (Porazinska et al., 2009; Yeates, 1987, 1999).

Carex parallela, monocot, is known as perennial herb growing in

moist places, mossy mats and has fibrous root. Silene acaulis, dicot, is

perennial and tends to form solitarily having tap root. It grows in a range

of substrates. (이유경 et al., 2012)

It is known that the roots of dicots and monocots of same biomass can

have different rate of water and nutrient uptake (Chapin III et al., 1975;

Hamblin and Tennant, 1987; Mengel and Steffens, 1985).

However, NMDS did not reveal any difference between the

rhizosphere communities of the two cushion plant species. Apparently the

environments underneath the two plant species are too similar, or (to put it

another way) the soil Metazoa are generalized enough, to produce a

difference in the communities. Partly this may be because potential

specialists (plant parasitic nematode families) were not the dominant

groups in the Svalbard rhizosphere environment. In fact, paradoxically,

plant parasitic nematode families were more abundant in cyanobacterial

mat areas of tundra: it is unclear why this may be the case, unless the

feeding behavior is different in arctic species of families that are normally

plant parasites in the temperate zone where they have been most studied.

If these nematode species tend to be bacterial or fungal feeders in the

arctic, it could explain why they are relatively more abundant in

cyanobacterial mat areas.

CHAPTER4. How do polar soil Metazoa communities differ from temperate ones? High polar tundra and temperate forest compared using a common methodology.

4.1 Introduction

One possible way of understanding polar ecology may be to compare its community structure, niche width and diversity in comparison to other ecosystems in warmer climates. Through such comparisons, the true nature of polar ecology may be better understood, and general theories of community structure and species coexistence may be arrived at.

Generally, there has been little study on small soil Metazoa (defined here as animals small enough to pass through the holes of a 2mm sieve) in polar environments. Although a range of phyla are known to occur (Maucci, 1996; PJA Pugh, 1998; Bale et al., 1997), most of the work that has been published is on nematodes (Coulson, 2013; Ruess et al., 2001). Even so, the nematode ecology of polar regions also appears to be the most poorly

understood of any terrestrial system, as many more studies have been done at boreal, temperate and tropical latitudes in both moist climate ecosystems and deserts (e.g. Shepard et al., 2002; Bloemers et al., 1997; Räty and Huhta, 2003; Salamon et al., 2006). This bias against polar nematodes (and polar soil Metazoa in general) is at least partly due to the practical difficulties of sampling and identifying such small animals in remote locations using traditional morphological criteria under a microscope.

In addition, due to lack of standardized comparable studies, it is unclear how the diversity and guild structure of polar nematode communities compares with those of lower latitudes. Boag & Yeates (1998) suggested that the global peak of soil nematode diversity lies not in the tropics but between 30 and 40 degrees N or S, in the mid latitudes, and reaches its lowest point in the high latitudes above 70 degrees N and S. That conclusion was at the time based on only two studies from the Arctic, and several from Antarctica.

It is also thought that the guild structure of polar nematode communities may be much simpler than in warmer climates. Studies from Antarctica's dry valleys and King George's Island reveal that plant feeders and specialized predators are mostly or entirely absent, and that the main nematode species present are bacterial feeders and omnivores (Mouratov et al., 2001).

In previous studies, over 500 species of arthropod have been recorded in Svalbard (Coulson 2007; Ávila-Jiménez et al., 2008). Interestingly, arctic arthropod diversity of Coleoptera and Diptera shows the reverse of the 'normal' pattern for lower latitudes in having 8.8 % of Coleoptera and 56.6% of Diptera ratio of the total species of Insecta (Coulson, 2007). Globally, by contrast, species diversity of Coleoptera is three times more than that of Diptera (Vernon et al., 1998; Chernov& Makarova, 2008).

Unlike most terrestrial arthropods of temperate latitudes, which tend to hide in microhabitats (e.g. under snow or in leaf litter etc.) to avoid

freezing temperatures in winter (Danks, 1991), polar arthropods have evolved freeze tolerance because freeze depth is greater (Danks et al., 1994)). Presumably, the same would be true for other small polar Metazoa, such as those I study here. With such unique features required for adaptation to extreme arctic environments, one can expect that small Metazoa would show distinct community patterns and distinct evolutionary lineages compared to those of temperate environments.

With a development of more sophisticated next generation sequencing methods, it became possible to analyze large scale databases. Using 18S nuclear small subunit rRNA gene, taxon richness is analyzed, and this reveals the diversity and community composition of soil Metazoa. Research conducted in tropical rainforest of Costa Rica using a metagenetic approach revealed that the nematode diversity and richness of tropical biome exceeded that of the temperate biome (Porazinska et al., (2010)). Also Poarzinska et al. found that contrary to previous consensus that nematode species show a cosmopolitan distribution due to their small

size (Baas Becking, 1934) nematode species tend to be distributed by a niche specific rather than ubiquitous pattern. This were also confirmed in a study carried out by Porazinska et al., 2012 which found that nematodes are not cosmopolitan, and show biogeographical patterns. They found 3-fold higher species richness of nematodes in the tropics than in the temperate biome.

However, while some clues have been obtained for nematodes, it is still unclear how metagenetic methods would change the overall global picture of the diversity and community structure of non-nematode soil Metazoa, such as arthropods, tardigrades, small annelids, Platyhelminthes, etc. The studies of Porazinska et al., 2010 have shown that nearly half the metazoan reads, and around 20% of species, obtained from soils in tropical and temperate rainforest were from these non-nematode groups.

In an analogous study comparing metazoan diversity in boreal forest and the arctic tundra by Wu et al. (2009), showed that the dominant phyla was Arthropoda in both region based on number of reads, having 79.7% of

boreal forest and 43% of arctic tundra.

Even before the advent of soil metagenetics, it was widely suggested that no more than 8% of the true diversity of mite species in the world had already been discovered (Shepard et al., 2002). Clearly, there is considerable potential for a new perspective on the soil meiofauna. So far, no standardized detailed ecological analyses have been carried out in any part of the world using metagenetics on these other non-nematode meiofauna — but the potential is there, if the same procedures for classification following online published reference sequences are followed.

The present study provides the first closely standardized inter-regional comparison of the diversity and community structure of Metazoa in the high arctic tundra, in relation to diversity and community structure in the temperate zone (Korea). Other past published inter-regional comparisons using morphological identification were limited by having used different methodologies for sampling and identification between the different climate zones. More closely standardized studies will yield

8 7

important clues to how the functioning of polar Metazoa communities

differs from (or remains similar to) these other biomes. Such comparisons

can start to give important clues to the workings of ecological communities

in general, and how they differ with latitude.

Furthermore, this study provides a 'testing ground' for the application

of metagenetic techniques to metazoan ecology in polar environments,

potentially opening up a wide range of research on a range of small animals

in soil, sediment and seawater (e.g. nematodes, mites, rotifers, tardigrades

etc.).

The present study was structured around investigating the following

questions: Is there evidence of a strong latitudinal difference in soil

Metazoa alpha- and beta- diversity between the high arctic (Svalbard)

and the temperate zone (Korea), paralleling what is found for many other

groups across wide latitudinal differences? I predicted that Svalbard would

have much lower alpha and beta diversity, in line with what is found for

most other groups of organisms, such as trees, birds and mammals (Lyons

and Willig, 1999). Is there evidence of a strong degree of species overlap between the high arctic and temperate zone, reflecting highly generalized niches in small soil Metazoas? Each species has a definable ecological range in terms of climate and geography (Dumbrell et al., 2009; Vandermeer, 1972). For larger organisms such as plants and mammals, there is little or no overlap in terms of the species present between high arctic and lowland temperate environments – the exception being birds which have definite summer–winter migration patterns between the two environments (Johnson and Herter, 1990). I predicted that due to the very different environmental requirements for survival in temperate and polar regions, there would be little or no evidence of species overlap. Is there a difference in trophic guild structure of soil Metazoas between Svalbard and Korea, possible reflecting fundamental differences in community and ecosystem functioning? It has long been thought that in warmer, moist climates, with greater primary productivity and less extreme physical conditions relative to the optimum temperatures for cellular processes,

specialized feeders on larger living organisms (e.g. parasites, specialized

predators, top predators) will be more diverse and abundant (Pianka,

1966). In extreme environments with low productivity, it is supposed that

resource supply is less predictable, preventing specialization by predators

and parasites, and food web structure is likely to be simpler with fewer

specialized parasites and predators.

By this principle, I predicted a simpler trophic structure in Svalbard with

fewer predators and plant parasites, and more microbial feeders or plant

decomposers.

4.2 Methodology

4.2.1 Sampling sites and times

A set of 5 samples from a temperate region site were taken 20-60m apart

in an interrupted grid in mid-August, 2013 in a natural old growth mixed

cool temperate Quercus, Castanea and Acer forest at Gwanak, South Korea

(latitude 37 N, longitude 25.425 E). Mean annual temperature of this site

is about 9.5 DegC, with cold winters (Mean January temperature −3 Deg.C), and warm wet monsoonal summers (Mean July temperature 25 Deg.C, with daily highs around 30 Deg.C).

Three sets of 5 arctic samples were taken in 3 identical interrupted grids near the Dasan Base in Svalbard in late July, 2013. On Svalbard, I chose 3 different tundra types based on vegetation cover: HV (High vegetation cover: >90% plant cover, latitude 78° 55′ 20.0″ N, longitude 11° 56′ 30.3″ E), IV (Intermediate vegetation cover: 70~85% plant cover, latitude78° 55′ 18.55″ N, longitude 11° 51′ 52.63″ E), LV (Low vegetation cover: <50% plant cover, latitude78° 55′ 58″ N, longitude 11° 49′ 22″ E).

The arctic site is high arctic tundra on Svalbard at 78° 55′ N. Mean annual temperature is −6.1° C measured in Svalbard airport, Longyearbyen during the period 1976−98(Isaksen et al., 2001), and temperatures are below freezing most of the year except a brief summer season when mean daily highs are around 5−8 ° C measured in 1993

91

(Oehme et al., 1996).

4.2.2 Sampling method

5 samples were taken from each tundra type: in total 15 samples were sampled in the arctic region, in three interrupted grids of 5 samples each. In the temperate region, I took 5 samples in a single interrupted grid. Each individual sample consisted of a 1m x 1m quadrat (Fig. 11). Scoops of soil of 200g were taken at 0–5cm depth in the B horizon of each soil after removal of surface litter (a clear A horizon was not present in any of the soils), using a sterile trowel. 5 scoops were taken in each quadrat, one at each corner and the other in the center. The 5 scoops of soil were placed in a sterile bag, taken immediately (with an hour) to a laboratory for extraction. The soil was gently mixed by kneading the bag, before extraction of small Metazoa began.
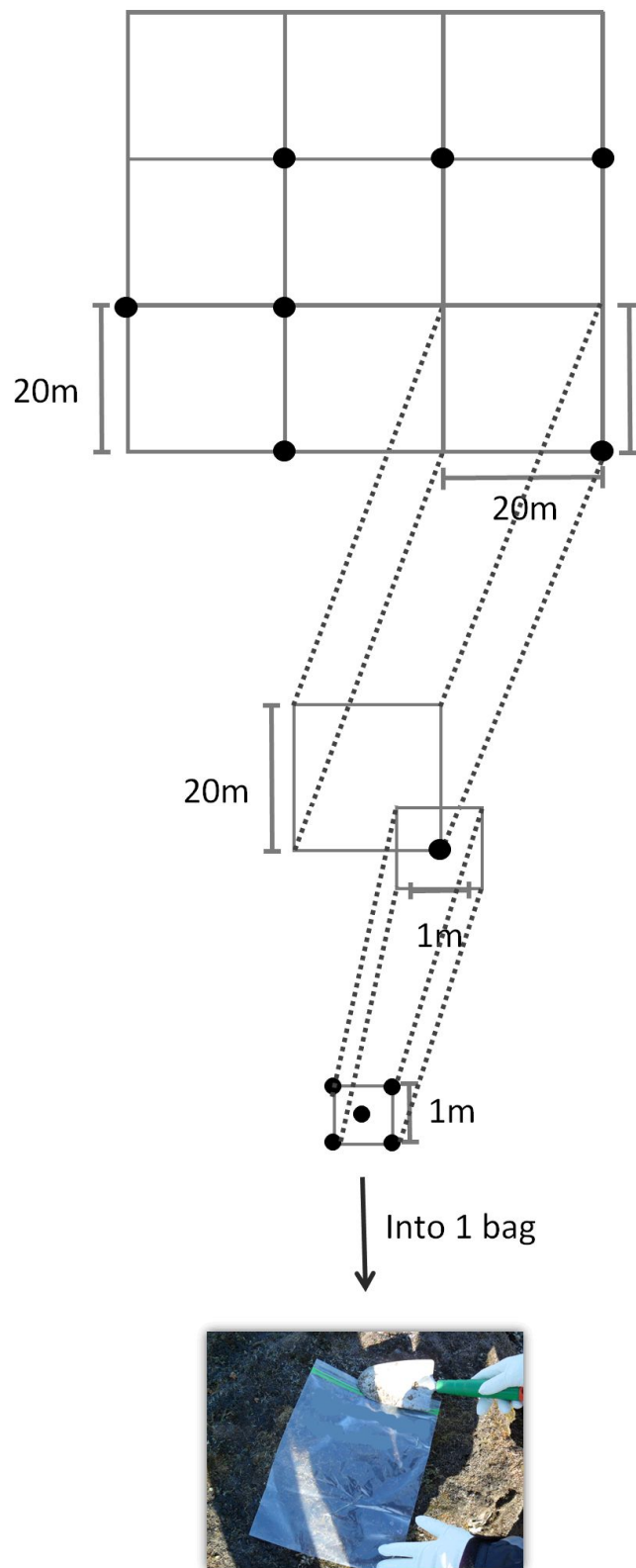
Fig. 10 Interrupted grid sampling system used in this study.

4.2.3 Soil Metazoa DNA extraction

Soil Metazoa were extracted with a combination of Baermann funnel (Thorne, 1961) and sugar flotation (Jenkins, 1964). The Baermann funnel technique, which is intended to capture live soil Metazoa, particularly nematodes, was carried out by loading 100g of the mixed soil into a funnel after removing small stones and plant debris. Active Metazoa moving down through the funnel sank and were collected in the tube below. After 24 hours, the fluid in the tube was let out and centrifuged at 3000 RPM to concentrate metazoan material as a pellet.

To capture less active metazoan material (e.g. slow moving animals, eggs and dead animals) the sugar density flotation method was adapted from Porazinska et al. (2012). The remaining soil from the funnel was placed in 40% sugar solution, gently stirred, and allowed to settle. The solution was centrifuged again for 30 seconds at 2000 rpm and the supernatant poured over a 38um sieve. Again with the help of a wash

bottle, the sediment was rinsed on top of the sieve and then collected into

a 50ml tube. The tube was centrifuged for 10min at approximately

3000rpm to settle metazoan material into the bottom and without disturbing

the pellet, the water was decanted.

4.2.4 DNA extraction, PCR and pyrosequencing of 18SrRNA gene.

Pellets captured from Baerman funnel and sugar flotation were separately

used to extract DNA by using MoBio PowerSoil DNA isolation kit according

to the manufacturer's instructions. Equal portions of both extracted DNAs

were then combined and used for PCR amplification. The primer I used was

defined by NF1 (C.elegans numbering 1226-1250) and 18Sr2b (C.elegans

numbering 1567-1588) towards the 3' end of the 18S rDNA

(Porazinskaet et al. 2009) which generates an amplicon of a ~400bp

diagnostic region. Amplified DNA samples were purified then

pyrosequenced with a 454 GS-FLX Titanium system (Roche).

4.2.5 Sequence processing.

I followed the Mothur 454 SOP pipeline (Schloss et al., 2009) using the SILVA SSU database was used to process generated sequences from pyrosequencing.

Generated sequences were processed following Mothur's 454 SOP, utilizing the NCBI database for both alignment and classifying commands. Briefly, sequences were denoised and processed using the Mothur pipeline (Schloss et al., 2009), which includes quality checking, aligning against SILVA SSU eukaryotic aligned database and chimera detection by UCHIME. Taxonomic classification of Metazoa was performed against the SILVA SSU database at a cut-off 50%. All singletons were removed before statistical analysis.

4.2.6 Statistical analysis.

Sequences were standardized per sample following sub.sample command ((http://www.mothur.org/wiki/Sub.sample) in Mothur which ended up with 856 reads of Metazoa and 432 reads of nematode. Operational taxonomic unit (OTUs) were obtained at 99% similarity processed from Mothur platform (Schloss et al., 2009). Diversity indices used for α-diversity comparison between biome was also processed by Mothur platform (Schloss et al., 2009). For such comparison was analyzed using pairwise comparison. For the normal distributed variables, an ANOVA test was performed and the Kruskal-Wallis test was used for non-normal distributed variables. To analyze β-diversity, both community β-diversity (Anderson et al., 2011) and true β-diversity (Koleff et al., 2003) were used. Using a community dissimilarity-based metric based on OTUs, variation in community (community β-diversity) was represented as average distance from group centroid to each sampling point was measured using betadisper function in R. True β-diversity was calculated

through following equation : $S/\bar{\alpha} = (a + b + c)/[(2a + b + c)/2]$; S: total

number of OTUs of two samples, $\bar{\alpha}$: average number of OTUs of two sample,

a: shared OTUs of two samples, b: OTU only found in sample1, c: OTU

only found in sample2. Then these values were compared by pairwise

comparison with Post hoc Tukey test. % Relative abundance was compared

at class level of different soil Metazoa and family level of different

nematode feeding group. For later, I chose top 15 abundant taxa and

grouped them by their feeding preference based on Yeates paper (1998).

Such comparisons were also performed through pairwise comparison using

either ANOVA or kruskal-wallis test. To see if the two biomes have

distinct community composition either soil Metazoa whole or nematode and

arthropod solely, community similarity was analyzed through unweighted

UniFrac then visualized into non-metric multidimensional scaling plots

(NMDS). This was further confirmed with an ANOSIM test based on

sequence difference between samples on the ground of phylogenetic

information. These were performed by using primer6 (Clarke and Gorley,

2006). Shared OTUs and indicator OTUs were calculated by Mothur platform. To reveal if any environmental variables including pH, TN, TOC and P_2O_5 delimiting community composition of two biome, I used the envfit function in package Vegan in R version 3.0.1.

4.2.7 Soil analysis.

Soil pH, total nitrogen (TN), available phosphorus (P_2O_5) and total organic carbon (TOC) were measured at National Instrumentation Center for Environmental Management (NICEM, South Korea) based on the standard protocol of SSSA (Soil Science Society of America).

4.3 Results.

4.3.1 General findings.

In the 15 arctic samples, I found 499 different Metazoa OTUs and 314 Nematoda OTUs across all three tundra types. In our smaller set of five samples from the temperate region, I found 614 Metazoa OTUs and 312 Nematoda OTUs. In total, 67130 reads were obtained from the Mothur platform and among those, 47,047 reads were designated as soil Metazoa which take about 70.08% of total reads. 26,803 reads were assigned as Nematoda, constituting 56.97% of total Metazoa.

4.3.2 $\alpha -$, $\beta -$ diversity and community composition in the arctic and temperate biome.

Pairwise comparison revealed that $\alpha -$diversity using OTU richness, Chao and ACE diversity index was significantly higher in the Korean temperate biome samples than in any of the sets of Svalbard arctic samples ($p < 0.05$). For Metazoa in general, individual temperate biome samples had almost three times higher OTU richness than individual arctic samples (Fig. 12). This was also true for nematodes (Fig. 12).

Conversely, the temperate region showed significantly lower Metazoa/nematode beta diversity, both community $\beta -$diversity and true $\beta -$diversity, than IV (Intermediate vegetation) tundra and LV (Low vegetation) tundra, but it did not a show any significant difference from HV (High vegetation) tundra ($P < 0.05$) (Fig. 13).
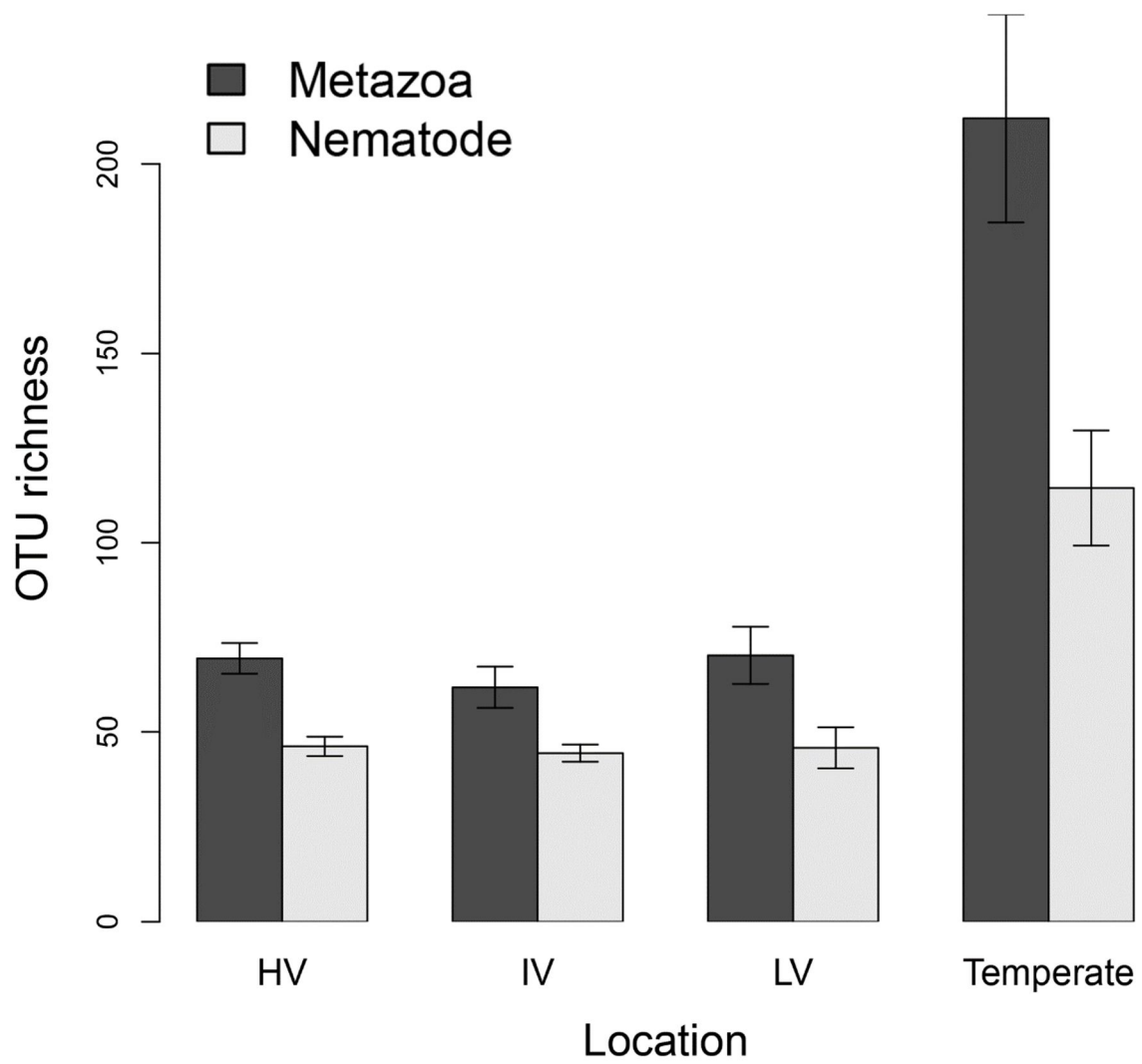
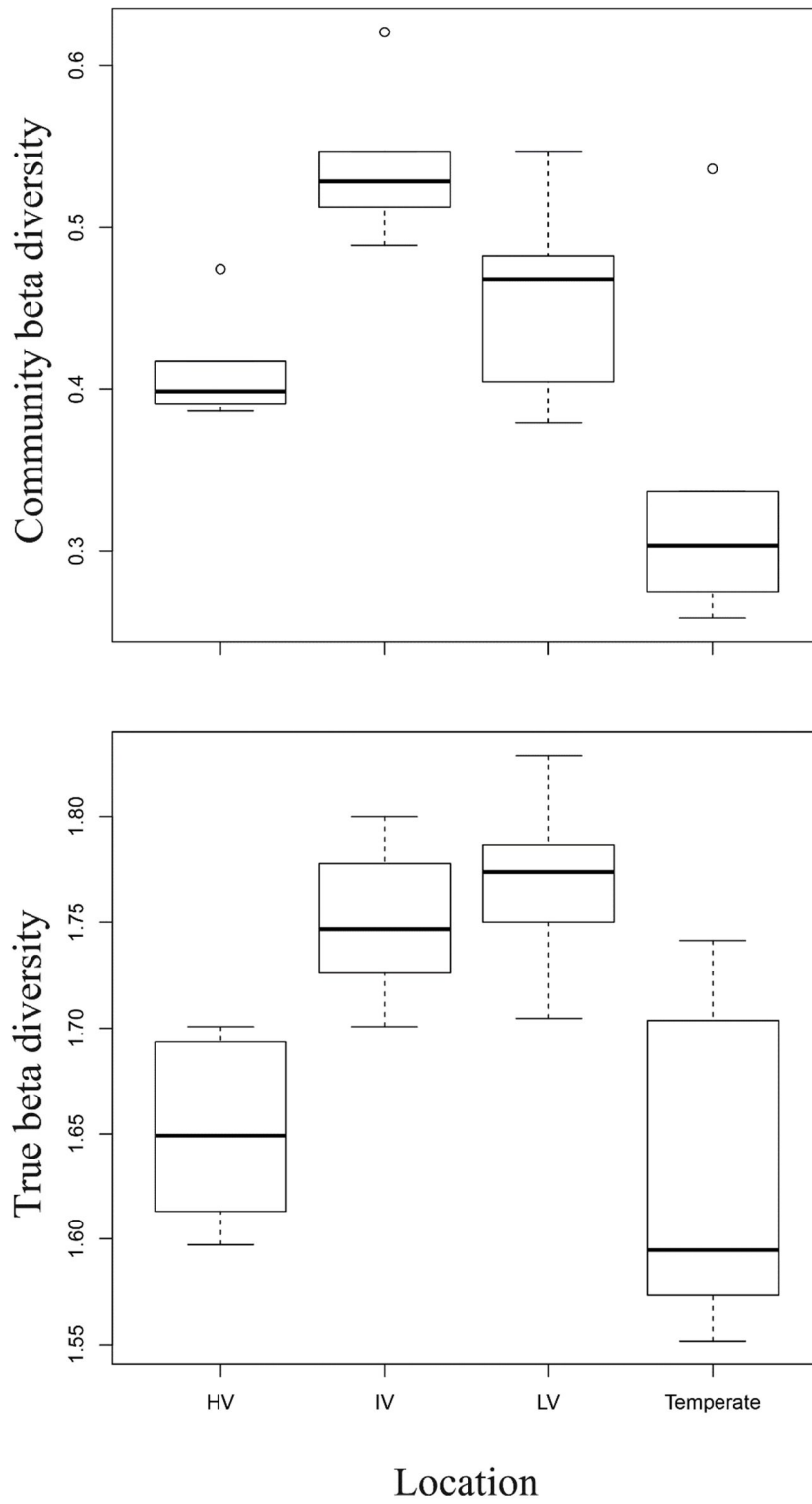Fig. 12 OTU richness of soil Metazoa and nematode in different locations.

Fig. 13 β -diversity of Metazoa in arctic tundra and temperate biome.

4.3.3 Community composition.

Comparison across the biomes of the relative abundance based on number of reads from different Metazoa groups showed that nematode and arthropod relative abundances were significantly different only between HV tundra and the temperate region. All three tundra types were significantly different compared to temperate region in terms of annelid abundance ($p<0.05$). In case of Nematoda, groups classified as predators were more abundant by numbers of reads in the temperate region than HV tundra and reads of bacterial feeding Nematoda were more abundant in the temperate region than LV tundra ($p<0.05$).

NMDS based on UniFrac distance showed that the community of all Metazoa combined was distinct between the arctic and temperate regions (Fig. 14). The nematode community was also significantly different (Fig. 15). This was further confirmed by an ANOSIM test ($p<0.05$). Metazoan community was distinct between all of arctic tundra and temperate biome (ANOSIM: Temperate-HV: R=1, *P <0.05; Temperate-IV: R=1, *P <0.05;

Temperate-LV: R=0.988, *P <0.05; HV-LV: R=0.264, *P <0.05).

Nematode community revealed same pattern (ANOSIM: Temperate-HV: R=0.992, *P <0.05; Temperate-IV: R=1, *P <0.05; Temperate-LV: R=0.888, *P <0.05; HV-LV: R=0.304, *P <0.05).
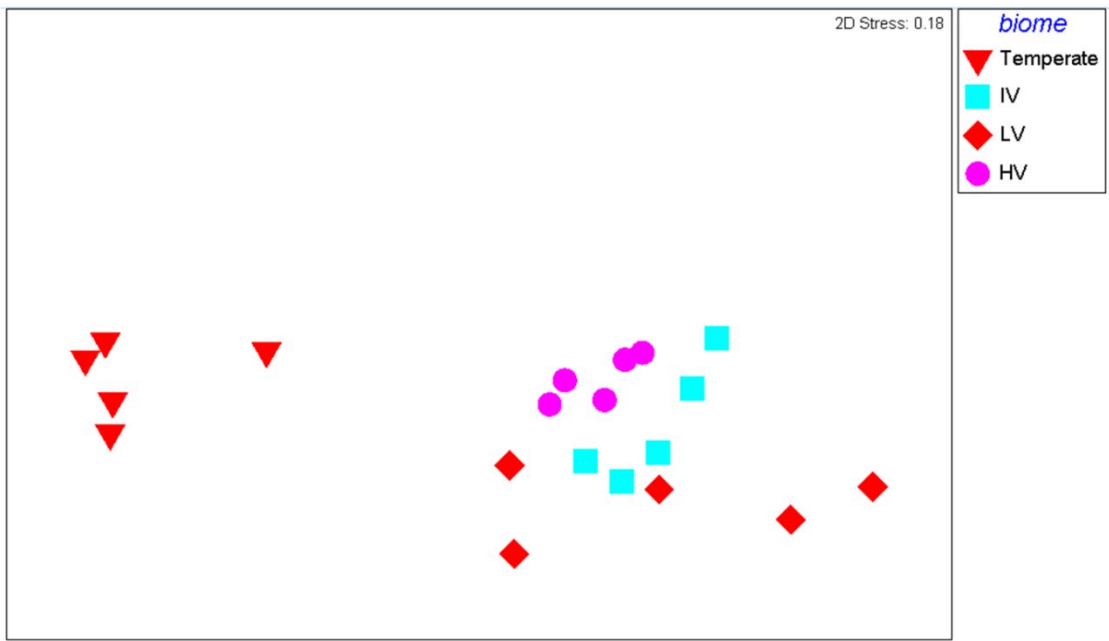
Fig. 14 NMDS based on UniFrac distance of total Metazoa community of

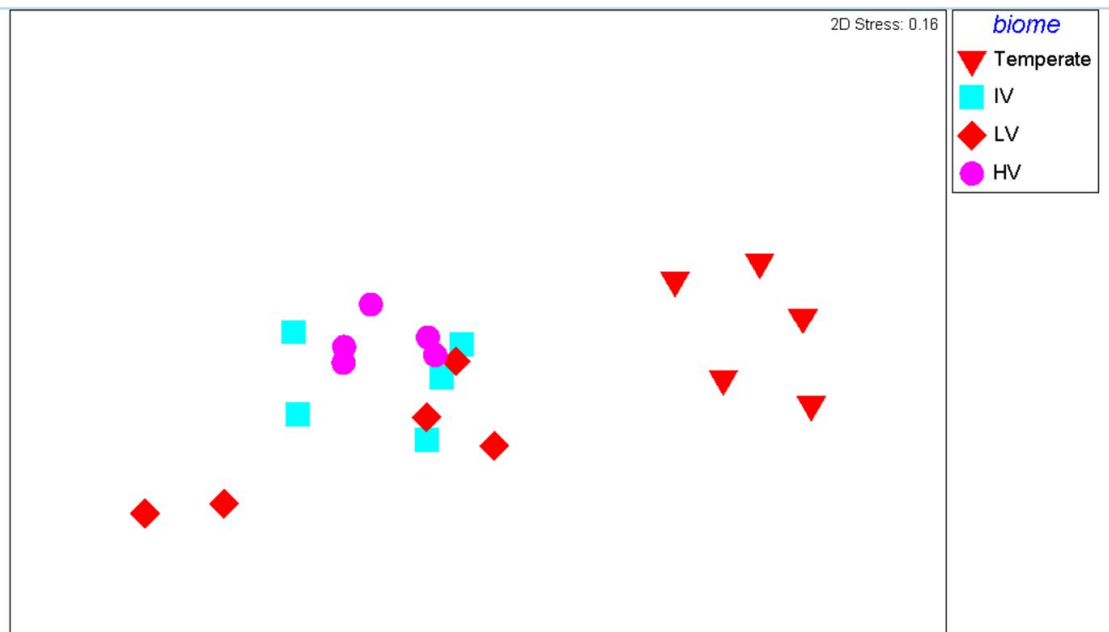temperate forest and arctic tundra.

Fig.15 NMDS based on UniFrac distance of Nematoda community of

temperate forest and arctic tundra.

4.3.4 Shared OTUs and Indicator OTUs

I compared corresponding sets of 5 samples from the tundra and the

temperate zone.

Of the 215 Metazoa OTUs in the 5 IV tundra samples and 614 OTUs

in 5 temperate zone samples, only 35 OTUs were present in both the arctic

and temperate sample sets (Fig. 16). For nematodes alone, the 5 samples

from the temperate biome had twice as many OTUs as a set of 5 arctic

samples from IV tundra. Of the 156 nematode OTUs detected in the IV

tundra in Svalbard and the 312 detected in Korea, 19 overlapped (Fig 17).

When the other tundra types were compared with the Korean

temperate forest, the general pattern was the same. HV tundra harbored

215 Metazoa OTUs and of these 48 OTUs overlapped with the temperate

biome. Out of a total of 248 Metazoa OTUs found in the 5 samples of LV

tundra, 41 OTUs overlapped with the 5 temperate region samples. In case

of nematode OTUs, 135 OTUs were found in the arctic and 24 OTUs were

shared with temperate region. Also LV tundra harbored 157 nematode

OTUs, with 24 OTUs shared with temperate region.

Overall, I found 499 metazoan OTUs in the 15 arctic samples and 614 OTUs in the 5 temperate biome samples. In the case of nematodes, 314 OTUs were found in the arctic whereas 312 OTUs were detected in temperate region. In this case, however, the greater total number of samples in the arctic masks the lower alpha diversity per sample compared to the temperate zone.

Based on OTUs, only four Nematoda families — Qudsianematidae, Dolichodoridae, Plectidae and Tylenchidae — were found principally in the arctic, whereas most taxonomic groups of Nematoda corresponding to species (OTU) up to family level were only found in temperate region (Table 6). Likewise, many other identifiable families of Metazoa including members of Arthropoda, Tardigrada, Rotifera and Annelida were confined to the temperate zone samples (see Table 6).
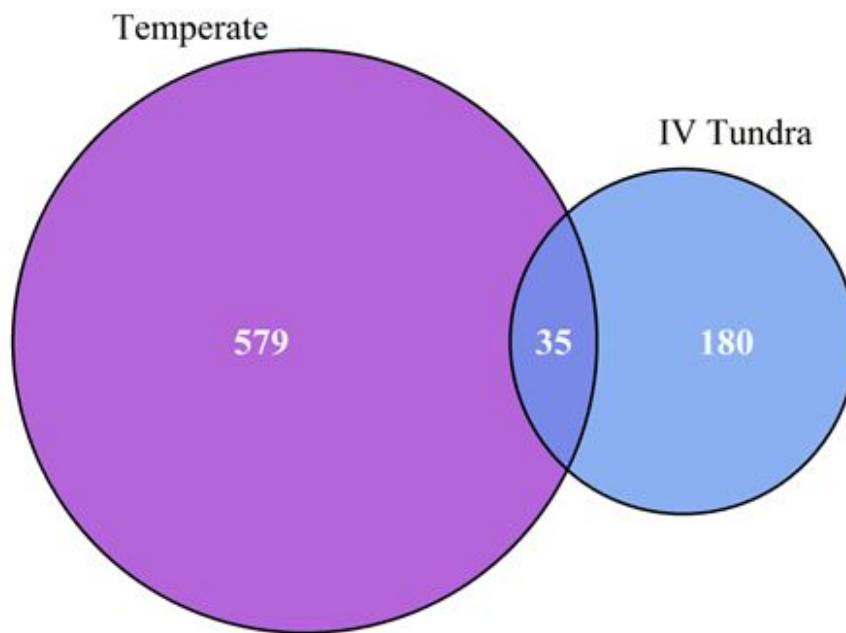
Fig. 16 Shared OTUs of all Metazoa compared between the 5 arctic

samples (Svalbard IV Tundra) and 5 temperate samples (Korean forest).

The 5 temperate region samples harbor a much higher number of metazoan

OTUs (species) than the 5 arctic samples, and only 35 OTUs are shared

between the two regions.
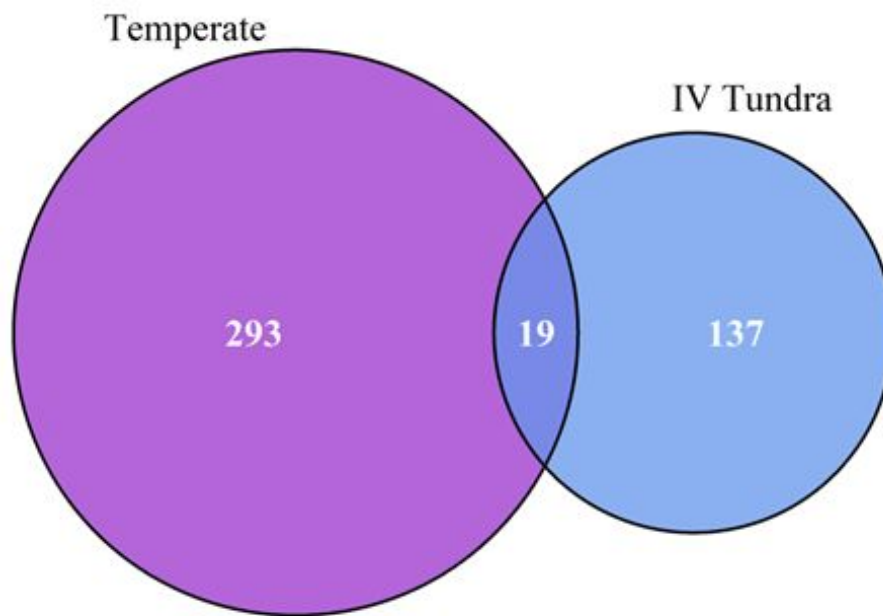
Fig. 17 Shared OTU (species) of Nematoda, compared between temperate
(Korean forest) and arctic (IV tundra) region. The 5 temperate region
samples harbor more than twice the number of species of the 5 arctic
region samples and only 19 species overlapping.

4.3.5 Soil properties structuring community composition.

Envfit function revealed that pH, available phosphorus and total organic

carbon were the factors delimiting Metazoa community.

Table 6 Indicator OTUs soil Metazoa from temperate biome and arctic tundra.

Biome	Indicator OTU class	Indicator OTU family	Indicator OTU SILVA SSU hits	Indicator Value	pValue	Size
Arctic	Nematoda	Enoplea	Qudsianematidae	>98	<0.02	14050
Arctic	Nematoda	Chromadorea	Dolichodoridae	>99	<0.03	8238
Arctic	Nematoda	Chromadorea	Plectidae	>91	<0.03	3878
Arctic	Nematoda	Chromadorea	Tylenchidae	>97	<0.02	1922
Temperate	Annelida	Annelida	Family_Incertae_Sedis	>94	<0.02	5029
Temperate	Rotifera	Rotifera	Philodinidae	>82	<0.03	1833
Temperate	Nematoda	Chromadorea	Tylenchidae	100	<0.02	383
Temperate	Arthropoda	Chelicerata	Arachnida	100	<0.02	230
Temperate	Arthropoda	Hexapoda	Insecta	100	<0.02	204
Temperate	Annelida	Annelida	Incertae_Sedis	100	<0.02	185
Temperate	Nematoda	Enoplea	Prismatolaimidae	100	<0.02	88
Temperate	Tardigrada	Hypsibiidae	Isohypsibius_papillifer	100	<0.02	85
Temperate	Nematoda	Enoplea	Prismatolaimidae	100	<0.02	84
Temperate	Nematoda	Chromadorea	Anguinidae	100	<0.02	74
Temperate	Nematoda	Enoplea	Mononchidae	100	<0.02	58
Temperate	Nematoda	Enoplea	unclassified	100	<0.02	54
Temperate	Nematoda	Enoplea	Tripylina_sp._SAN-2007a	100	<0.02	34
Temperate	Nematoda	Chromadorea	Aphelenchida	100	<0.02	29
Temperate	Nematoda	Enoplea	Dorylaimidae	100	<0.02	13

4.4 Discussion.

4.4.1 Metazoa alpha diversity of Svalbard tundra is less than that of Korean temperate forest, but the tundra has higher beta diversity.

I compared our tundra diversity results with meter-scale samples, on a interrupted grid, from Korean temperate forest (Gwanak Mountain), arranged in the same grid pattern as our tundra samples (20m spacing). The total total Metazoa and Nematoda communities of the Korean forest samples were significantly more diverse at the alpha diversity level. However, the Svalbard tundra samples overall were more diverse at the beta diversity level.

It appears that for soil Metazoa, α-diversity may follow the 'classic' latitudinal trend seen for so many other groups of organisms. A review based on earlier work, by Bongers (1998), suggested that polar nematode communities may be less diverse than temperate zone ones, but this was based on only three studies of polar regions, without standardized

113

sampling in both regions. The greater sampling intensity enabled by

metagenetic techniques here appears to consolidate that finding, although

of course more studies would be necessary to conclusively confirm this

trend. In the next year of this study, I plan to take additional samples from

Korean temperate forests to compare with our Svalbard samples. I will also

compare soil Metazoa samples gathered from Malaysia and Brunei

rainforest – some already gathered and others being gathered in separate

projects in summer 2014.

However, it is unexpected to find that β−diversity is higher in two

of the three types of the Svalbard tundra (the more sparsely vegetated IV

and LV types), compared to the Korean temperate forest. This might in

part reflect the smaller scale patchiness of these tundra types, as opposed

to the broad uniformity of the forest dominated by large trees with thick

soil. However, it does offer an interesting novel perspective on the

latitudinal diversity gradient, and calls for further investigation.

4.4.2 There is little OTU overlap between Korean temperate forest and Svalbard tundra.

The indication is that there are very few cosmopolitan nematode species, capable of reaching from the temperate zone to the high arctic, but such species do nevertheless exist.

As an adaptation to the extreme low temperature environment of Svalbard, at least some species of nematode are cold-tolerant (Carlsson et al., 2013) and the need for such special physiological characteristics might be the reason for such distinct fauna in the arctic. In case of other Metazoa including nematodes, a much higher number of OTUs was detected in the temperate region (579 OTUs found only in the temperate zone) compared to only 180 OTUs (species) in the arctic, and with only 35 OTUs (species) overlapping between the arctic and temperate samples. This indicates that for non-nematode Metazoa, the contrast in diversity between the arctic and temperate zone is greater than for Nematoda.

4.4.3 Nematode guild structure is different between the Korean and Svalbard sites.

The most significant difference between the two regions was in Annelida, where the percentage of reads was always significantly lower in the tundra than in the temperate region. Nematoda and Arthropoda were only significantly lower in relative abundance in HV tundra than in the temperate region. It appears that the temperate the environment provides a favorable habitat for a wider range of soil Metazoa even though nematodes were the dominant phyla in both biomes. Another similar study, (Wu et al., 2009) compared soil Metazoa diversity in boreal forest and arctic tundra using 18S rRNA 18S11b (5′-GTC AGA GGT TCG AAG GCG-3′) and the reverse primer 18S2a (5′-GAT CCT TCC GCA GGT TCA CC-3′). They also found relatively more reads of Nematoda in the arctic region, and only Annelida, Arthropoda and Mites had higher relative abundance in boreal forest than arctic region. Porazinska's (2012) work which compared

nematode diversity and community composition between temperate and

tropical region, revealed that the temperate and tropical biomes have very

distinct nematode community (no shared nematode OTUs between the two

biomes) and that the tropical region harbored 300% greater richness of

Nematoda OTUs than the temperate region. From this combined picture, I

can argue that even if small soil Metazoa are widely distributed

geographically, they follows the classic latitudinal diversity gradient – with

clear differentiation of taxa by climate zones – rather than cosmopolitan

uniformity.

CONCLUSIONS

There is evidently an important element of niche and habitat differentiation amongst small soil Metazoa between the three different tundra types, producing distinct communities. It appears that even in this 'extreme' environment, many small soil Metazoan species are to some extent habitat specialists rather than generalists – having greater abundance and most consistent presence in one type of tundra than another. Shannon α – diversity may be promoted by carbon and P availability, while β –diversity may be promoted by patchiness in vegetation cover and associated environmental differences.

As with earlier studies, it is clear that there is niche differentiation within the Metazoa community among these very small-scale environments and the differences contribute to the overall diversity. As one would predict, the presence of plants and cyanobacterial mats increases diversity, possibly through modification of the soil chemical environment.

However, I found no evidence of distinct soil Metazoa and Nematoda

118

communities under the two taxonomically very distinct cushion plant species, suggesting that both species of plant are ecologically equivalents for the small soil fauna. Lack of soil chemical differences within the rhizosphere of these plants as well as similarity in their root characteristics would explain similar metazoan community composition underneath them.

We found that total nitrogen, total organic carbon, available phosphorus and pH may delimit Metazoa community composition of different microsites, perhaps because more abundant resources allow a greater range of specialized niches to exist at viable population densities. In case of rhizospheric Metazoa, total nitrogen and pH in particular have positive correlation with diversity.

It appears that soil Metazoa may show the 'classic' latitudinal alpha diversity trend, being less diverse in tundra than in temperate forest. However, beta diversity may be similar or greater in tundra due to the

small scale mosaic of microhabitats. This may however be merely a product of high percentage turnover: total gamma diversity is lower in the sets of 5 arctic samples. Especially high beta diversity in intermediate-vegetation cover (IV) tundra may be favored by a more diverse mosaic of microhabitats.

As a more general point, the metagenetic method used here appears to offer considerable promise for studying small metazoan communities in polar regions. With relatively little labor input, a large number and wide range of taxa was detected – more so than in any previous study of Svalbard. Their degree of overlap with known soil Nematodes from previous studies of Svalbard suggests that, as in metagenetic studies elsewhere, taxonomic assignment of reads is reliable.

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Ecosystem-Level Effects of Enriched Plant Diversity. Functional Ecology, 410-416.

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Supplementary

List of Supplementary

Fig. S1 β diversity of Metazoa in three different tundra shows that IV tundra harbors higher β diversity than HV tundra ($P < 0.05$)

Fig. S2 NMDS based on UniFrac distance of Metazoa in three different tundra shows that HV and LV tundra harbors distinct Metazoa community.

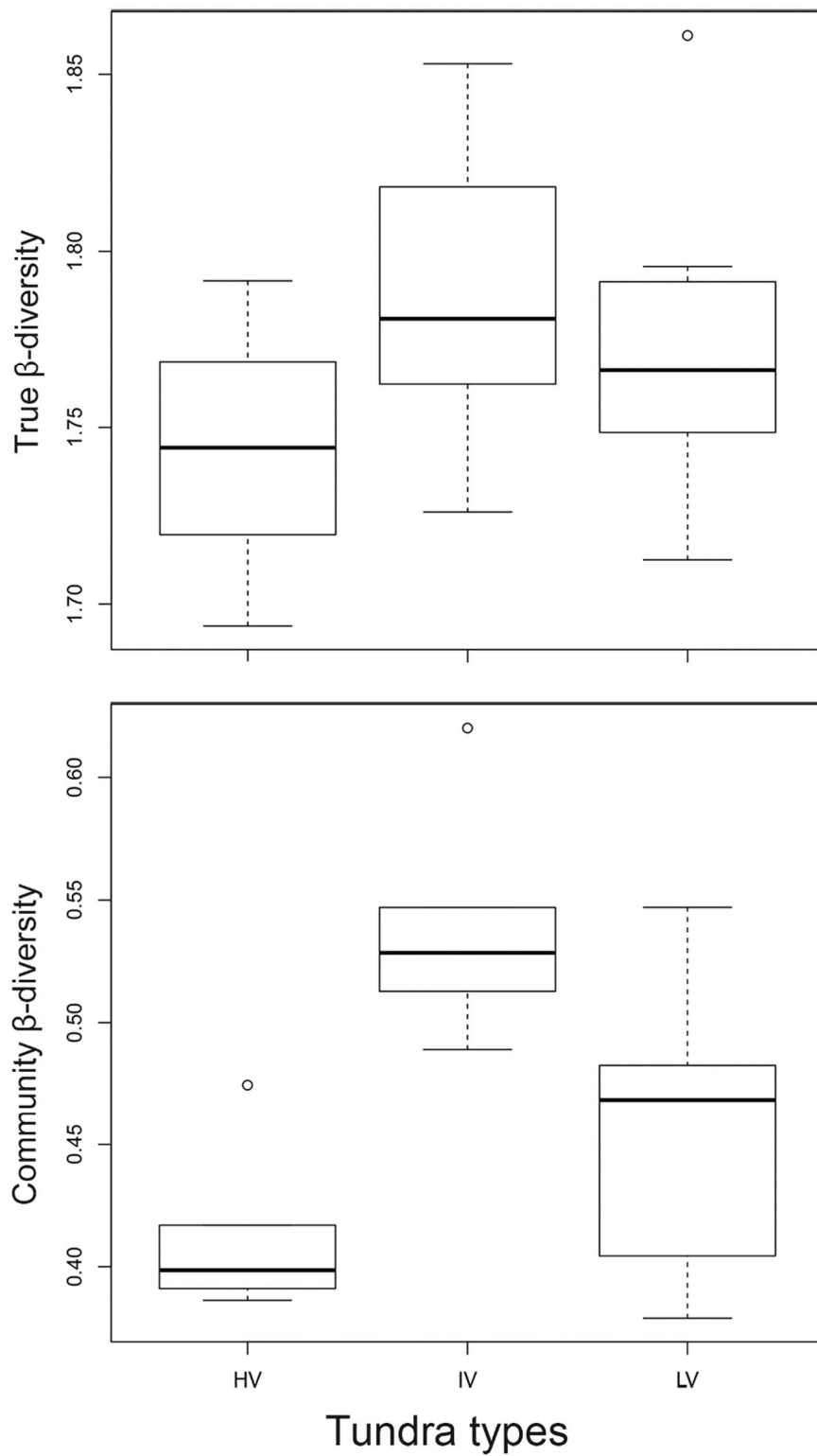**Fig. S3** NMDS based on Euclidean distance of three different tundra.

Fig. S1 β diversity of Metazoa in three different tundra shows that IV

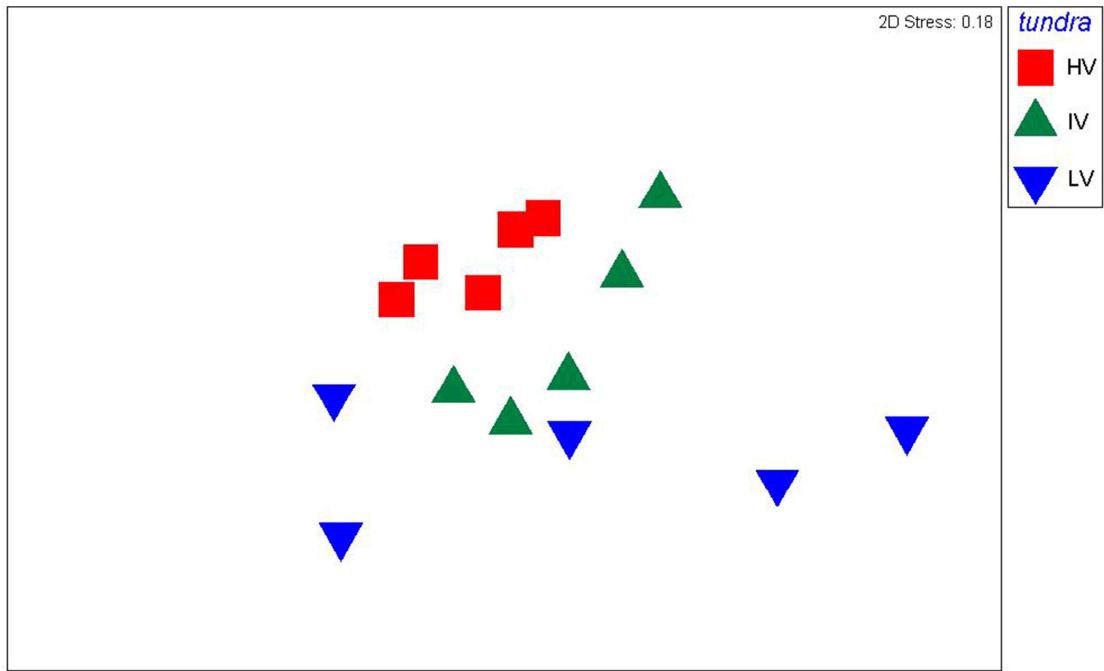tundra harbors higher β diversity than HV tundra (P < 0.05)

Fig. S2 NMDS based on UniFrac distance of Metazoa in three different

tundra shows that HV and LV tundra harbors distinct Metazoa community.
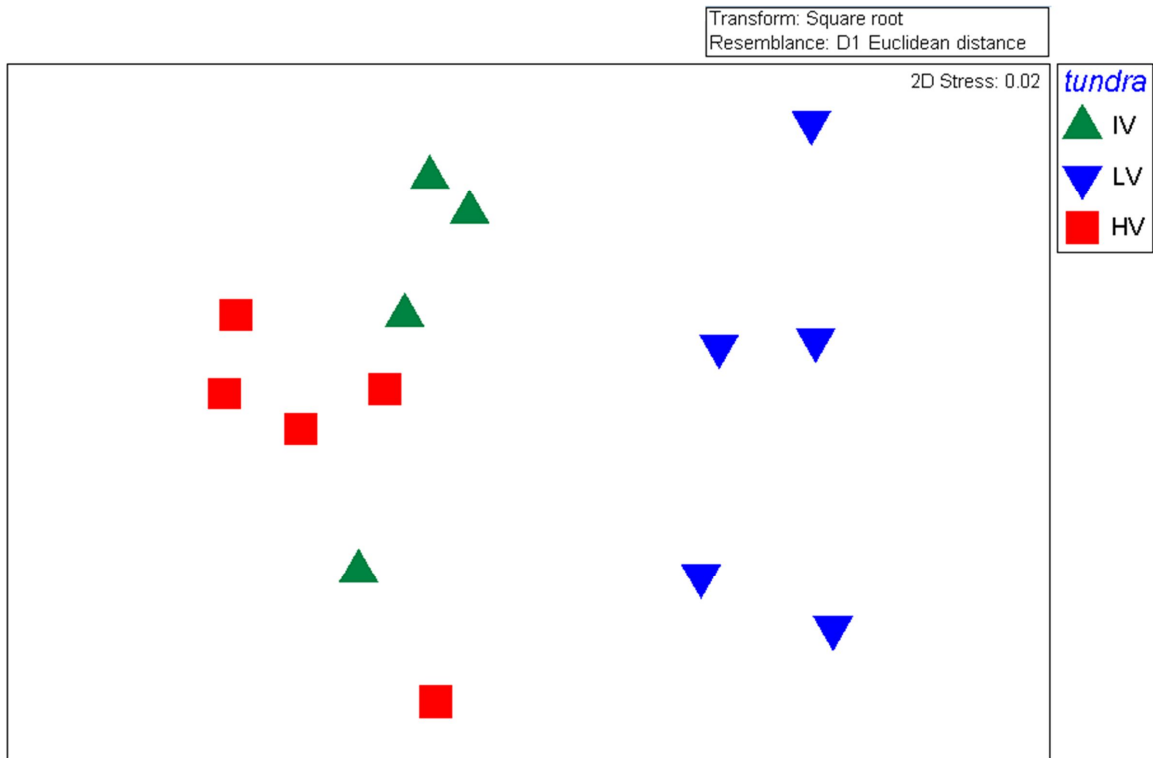
Fig. S3 NMDS based on Euclidean distance of three different tundra.

국문초록

이 연구의 목적은 메타지노믹스를 이용해 극지의 토양미소동물 군집에 관한 아래 질문들의 답을 찾는 것이다. 1) 토양미소동물 군집의 구조와 다양성이 툰드라(스발바드), 온대(한국)지역에서 위도에 따라 어떻게 달라지는가? 2) 포식 방법에 따른 토양미소동물 우점종이 위도의 변화에 따라 달라지는가? 3) 특정 식물 또는 토양에만 분포하는 고유의 토양미소동물 군집이 툰드라에도 존재하는가? 4) 스발바드에서 토양미소동물 군집의 구조와 다양성이 천이 과정에 따라 어떻게 변화하는가? 5) 근권, 돌 아래, 황무지 등과 같은 툰드라의 다양한 미세입지에 따른 고유의 토양미소동물 군집이 존재하는가?

본 연구에서는 스발바드에서 생명활동이 가장 활발한 시기인 7월에 극지의 토양미소동물 군집 연구를 위한 시료를 채취하였다. 토양 표면으로부터 5cm 이내의 토양, 다양한 툰드라 식물의 근권, 낙엽층과 같은 다양한 미세입지에서 토양미소동물 군집 연구를 위한 시료를 채취하였고 이후 시료 채취 지점의 미세기상, 지피식물 유무 등과 같은 환경인자가 미소동물 군집에 미치는 영향도 분석하였다.

토양 미소동물 군집을 온전하게 추출하기 위해 깔때기법과 분별부유원심분리법을 조합해 토양시료로부터 활발하게 움직이는 토양 미소동물 그룹과 휴면중이거나 적극적으로 움직이지 않는 그룹의 토양 미소동물들을 모두 추출하였다. 깔때기법과 분별부유원심분리법을 통해 추출한 두 개의 DNA를 합쳐서 PCR의 template으로 사용하였다. primer는 NF1 (C.elegans numbering 1226-1250) / 18Sr2b (C.elegans numbering 1567-1588)를 사용해 18S rDNA의 분류를 위한 구간을 증폭하였다. PCR을 통해 증폭된 rDNA는 454 GS-FLX Titanium system (Roche)을 이용해 pyrosequencing 하였다. 결과 시퀀스는 denoising, trimming, chimera detection과 같은 엄격한 데이터 필터링을 거친 후 계통분류와 섭식 유형에 따른 분류를 수행할 것이다. MEGABLAST와 MUSCLE을 이용해 99% 유사도의 OTUs(Operational

Taxonomic Units)를 만들고 이를 바탕으로 또한 종다양성(species diversity; Phylotypes richness, phylogenetic diversity, Shannon diversity index), 베타 다양성(beta diversity)등 토양 미소동물 군집의 다양성과 풍부도를 나타내는 지수들을 산출하였다. 또한 고도, 기후 인자, 토양의 이화학적 특성, 지피식물과 같은 환경 인자들이 이러한 지수들에 어떠한 영향을 미치는지 분석할 것이다. 또한 토양 미소동물 군집의 다양성지수와 환경인자들의 상관관계를 알아보기 위해 다중회귀분석과 분산분석을 수행하였다. Unifrac을 사용해 군집 간 계통분류학적 유사도를 계산하였고 이후 이렇게 계산한 토양 미소동물 군집간 유사도를 Nonmetric multidimensional scaling (NMDS)을 이용해 시각화 하였다. 토양미소동물 군집의 구조와 다양성은 OTUs에서 문(phylum)까지 다양한 분류학적 수준에서 비교 연구 되었다.

또한 온대와 열대에서 이루어진 토양미소동물 군집에 관한 기존 연구를 바탕으로 위도에 따라 토양미소동물 군집의 구조와 다양성이 어떻게 달라지는지를 알아보고자 위도에 따라 생태자리의 너비가 어떠한 경향을 가지는지, 어떤 섭식유형이 우점하는지와 같은 생태학의 고전적인 가설을 토양미소동물에 적용하였다. 이 연구는 툰드라 생태계의 통합적인 기능을 이해하는데 중요한 기여를 하게 될 것이다. 1) 토양미소동물 군집이 툰드라의 다양한 서식지에서 어떻게 분포하는지, 또한 온대지방과는 어떻게 다른지에 대한 이해를 넓힌다. 2) 토양 미소동물의 생태자리가 어떻게 분화되는지를 알아봄으로써 서로 다른 종들의 공존과 생물다양성에 관한 이해를 넓힐 수 있다. 3) 토양미소동물 군집이 툰드라 생태계에 미치는 영향의 정도는 생태자리에 잘 특화된 종에 의해 좌우될 수 있다. 이 같은 연구를 바탕으로 토양 미소동물을 환경 보전과 복원의 지표로 활용할 수 있다.

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