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Complete List of Authors:	Zielińska, Sylwia; Univeristy of Gdansk, Department of Molecular Genetics of Bacteria Kidawa, Dorota; Univeristy of Gdansk, Department of Vertebrate Ecology and Zoology Stempniewicz, Lech; Univeristy of Gdansk, Department of Vertebrate Ecology and Zoology Łoś, Marcin; Univeristy of Gdansk, Department of Molecular Genetics of Bacteria Łoś, Joanna; Univeristy of Gdansk, Department of Molecular Genetics of Bacteria
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Environmental DNA as a valuable and unique source of information about ecological networks in Arctic terrestrial ecosystems

Sylvia Zielińska^{1*}, Dorota Kidawa², Lech Stempniewicz², Marcin Łoś¹ and Joanna M Łoś¹

¹Department of Molecular Biology, University of Gdansk, Wita Stwosza 59, 80-308, Gdansk, Poland, Present address: Department of Molecular Genetics of Bacteria, University of Gdansk, Wita Stwosza 59, 80-308, Gdansk, Poland

²Department of Vertebrate Ecology and Zoology, University of Gdansk, Wita Stwosza 59, 80-308, Gdansk, Poland

Correspondence: Sylvia Zielińska, University of Gdansk, Department of Molecular Genetics of Bacteria, Wita Stwosza 59, Gdansk 80-308, Poland, +48585236047, sylwia.zielinska@biol.ug.edu.pl

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Abstract

Arctic terrestrial ecosystems are particularly vulnerable to the effects of ongoing and predicted climate changes. The current states of environmental biodiversity and ecological networks in the Arctic need to be known and understood in order to monitor how they change and how these changes may influence the particular components of the ecosystem. Despite the fact that the Arctic tundra is generally poor in nutrients, it has a surprisingly high biodiversity, especially of invertebrates and microorganisms. Besides macroclimatic features, there may be local factors influencing biodiversity, such as microclimate, water availability or large seabird colonies depositing guano. This last can have a substantial impact on the soil's physicochemical features, and consequently the distribution, number and diversity of tundra-associated plants and animals in the vicinity of the colony. Changes in the Arctic biodiversity and the functioning of the ecosystem at all trophic levels are difficult to investigate using traditional methods. In this review, we discuss how modern molecular techniques, including

next generation sequencing, influence our ability to investigate and understand this ecosystem at both the micro- and the macro-scale and how they can complement the more traditional approaches to studying ecological networks in the Arctic.

Key words: Arctic, eDNA, metagenomics, environmental samples, faeces, soil

Introduction

The Arctic region comprises about 4.8% of the Earth's land surface north of the tree line, where the average temperature in July, the warmest month, is below 10 °C (Remmert 1980, Stonehouse 1989, Meltofte 2013). The Arctic comprises heterogeneous habitats created by gradients of geomorphology, latitude, proximity to coasts and oceanic currents. The physical characteristics of the Arctic important for structuring biodiversity include extreme seasonality, short growing seasons with low temperatures, the presence of permafrost, snow and ice cover. The high Arctic is dominated by bare, sparsely vegetated soils with a small number of plant and animal species and a short growing season. The low Arctic area is covered by mainly shrubby tundra vegetation (Remmert 1980, Stonehouse 1989). Species richness is generally low in the Arctic and tends to decline from the low to the high Arctic (ABA 2013, Meltofte 2013).

Short thaw seasons, low soil temperatures and low rates of evapotranspiration during the growing seasons in the Arctic severely constrain microbially mediated processes such as decomposition and nutrient mineralization, thus lowering rates of nutrient supply to plant roots. As a result, rates of plant growth and nutrient cycling between plants and soils are exceedingly low in terrestrial Arctic ecosystems. These are thus considered to be generally simple, with poor species diversity, and characterized by short food chains and a permanent shortage of nutrients. In fact, however, the biodiversity, especially that of cold-resistant species of invertebrates and microorganisms, is surprisingly high (e.g. Coulson 2007, Danks

and Smith 2009, Chu et al. 2010, Coulson et al. 2014, Koyama et al. 2014, Marusik et al. 2016, Zielińska et al. 2016c). Despite the extreme conditions, even glaciers can be a habitat for various microorganisms (Musilova et al. 2015). In cold environments, both soil and permafrost are dominated mainly by bacterial, archeal and fungal lineages (Makhalanyane et al. 2016).

The unique area of the Arctic is receiving much attention as a consequence of observed and predicted rapid environmental changes associated with global climate change (e.g. AMAP 2012, Gough et al. 2016, Makhalanyane et al. 2016). Although the last 10 000 years have been characterized by climatic stability, air and sea surface temperatures have increased markedly during the last few decades, and these changes are the fastest in the Arctic region (Hansen et al. 2010, GISTEMP Team 2016). Temperature, the most important environmental factor, has a strong impact on ecosystem structure and functioning, often contributing to a cascade effect (Cooper 2014). Arctic terrestrial ecosystems are highly sensitive, since even a small increase in temperature causes a decline in glacier ice and snow, and melting of permafrost (Callaghan et al. 1995, AMAP 2012, Gilg et al. 2012, Stempniewicz et al. 2016). Consequently, changes in Arctic biodiversity and the functioning of the ecosystem at all trophic levels, from microorganisms to vertebrates, are being observed and are expected to continue (e.g. ACIA 2007, Stempniewicz et al. 2007, Grémillet et al. 2009, Gilg et al. 2012).

The availability of liquid water is regarded as the most important abiotic stress factor influencing polar terrestrial communities. Precipitation occurs mainly as rain in the maritime zones during summer and inland as snow. However, temperature influences the rate of snow- and ice-melt, and eventually, levels of soil moisture, which is essential for the abundance of soil invertebrates and microorganisms. Increases in temperature and precipitation will affect ecosystems in a complex manner: longer growing seasons, thawing permafrost and changes in

the water cycle are expected to have the greatest impact on terrestrial ecosystems (Convey et al. 2014, Cooper 2014). Despite the fact that severe environmental conditions in the Arctic appear to be impeding the establishment and reproduction of alien species, rising temperatures and an increasing human presence are making it easier for new species, including parasites and other pests, to gain a foothold in the Arctic (Coulson et al. 2014).

Moreover, increases in vegetation productivity and climate feedbacks induced by shrub encroachment are expected to mediate changes in trophic interactions (Croll et al. 2005, Cooper 2014). Since the growing season in the Arctic is very short, even small changes in its duration will have consequences for most resident plants and animals. Plants may experience a direct effect in the form of increased growth, productivity and changes in community structure, which may provide better living conditions for herbivores. Climate change, however, may lead to a phenological mismatch between the timing of reproduction in herbivores, birds and mammals, and the availability of their food (Post and Forchhammer 2008, Moe et al. 2009). Non-migratory animals could be severely affected by changed winter snow conditions, the alternating melting of snow and freezing of the tundra surface known as “rain-on-snow events” that negatively affect the availability of plant food to herbivores. Such weather extremes in the Arctic may become more frequent in the future (Kohler and Aanes 2004, Vikhamar-Schuler et al. 2013, Descamps et al. 2016). Overall, the consequences of current warming for Arctic biodiversity may be serious and multifaceted, and hence difficult to predict (ABA 2013, Eamer et.al. 2013).

The aim of this review is to demonstrate how mutual relations between species as well as changes in ecosystems can be monitored faster and more easily using molecular approaches. This is particularly important in the case of polar ecosystems, given the conspicuous climate changes and the roughly three times faster temperature increase than in other regions of the world (ACIA 2007). Because of the extensive research area, harsh

conditions and environmental complexity, the proper identification and monitoring of Arctic biodiversity and ecological networks can be quite problematic. For instance, although faeces residues can indicate the presence of particular animals (Brinkman et al. 2010, Goode 2011), the structure, functioning and health of their populations remains unknown. The ongoing development of molecular tools, metagenomics and environmental DNA (eDNA) techniques means that we can obtain more detailed information about microorganisms, plants and animals based only on easily accessible environmental samples, like soil or faeces. A combination of soil microbiology and metagenomics, together with next generation sequencing (NGS) and eDNA techniques, could be used for a comprehensive analysis of a specific environment (e.g. Cowan et al. 2015, Makhalanyane et al. 2016). These molecular techniques can deliver a large amount of data concerning both micro- and macroorganisms and are useful for considering many ecological aspects of the Arctic ecosystem. Traditional ecological methods based on observation, collecting and trapping, though effective, are much more time-consuming than molecular approaches like metagenomics or eDNA analysis, which are often much easier to carry out and above all more quickly. Molecular techniques may thus be potentially valuable for monitoring the rapid changes taking place in the Arctic ecosystem.

Ornithogenic soils - a specific example of the Arctic environmental network

In general, the availability of nitrogen in the Arctic is limited because of low temperatures, low precipitation, slow chemical weathering of bedrock and poor soil aeration (Callaghan 1995, Wainwright et al. 1998). Arctic soils are permanently frozen (permafrost) except for a thin surface zone that thaws each summer. Besides environmental determinants like temperature and humidity, additional factors, such as the supply to the local tundra of marigenic nutrients by colonial seabirds and through sea spray can have a substantial impact

on the soil's physicochemical features (Oechel 1997, Zwolicki et al. 2013, 2016). However, such patterns of soil fertilization associated with higher species richness generally occur to only a very limited extent. A specific example is that of colonial seabirds, which forage at sea and breed on land, and deposit large amounts of organic matter (i.e. guano, eggshells, feathers, carcasses) near their colonies, enhancing the formation of ornithogenic soils (Stempniewicz 1990, Zwolicki et al. 2013, 2016). The areas adjoining seabird colonies constitute a rare oasis in Arctic habitats. The large-scale transport of organic and inorganic matter from the sea to the land supports diversification of local Arctic communities. Soil fertilization close to the seabird colonies facilitates the diversity and abundance of terrestrial plant communities and their accompanying fauna (Vidal et al. 2003, Stempniewicz et al. 2007, Zwolicki et al. 2013, Wojciechowska et al. 2015). The abundance of invertebrates, such as springtails (*Collembola*) or water bears (*Tardigrada*), that inhabit mosses, lichens, liverworts and soil, was found to be higher where soils had become ornithogenically enriched (Stempniewicz et al. 2006, Zawierucha et al. 2015, Zmudczyńska-Skarbek et al. 2015). Also, numbers and biomasses of each microorganism taxon and the whole structure of microbial communities can fluctuate in different soil environments, as a result of the changing physicochemical features of the soil (Aislabie et al. 2009, Grzesiak et al. 2015, Zielińska et al. 2016c).

Moreover, physicochemical soil parameters that impact the vegetation structure can be directly or indirectly associated with invertebrate and vertebrate diversity. A rich vegetation attracts populations of herbivores, and subsequently predators, scavengers and decomposers (Croll et al. 2005, Jakubas et al. 2008, Zmudczyńska et al. 2012). By their intensive grazing, herbivores make room for a more diverse group of plants in the ecosystem than just lichens (Van der Wal and Brooker 2004). Jakubas et al. (2008) showed that the abundance of herbivores associated with bird colonies depends on the size and location of the latter, which

in turn is correlated with the richness of the area near the bird colony. In addition, the presence of numerous herbivores and top predators in the area can also enhance soil nutrient concentrations (from urine, faeces and prey remnants (Gharajehdaghipour et al. 2016). By converting organic compounds into easily assimilated nutrients, faeces are a source of soluble nitrogen introduced into the environment. Figure 1 illustrates the theoretical ecological network associated with ornithogenic soil, developed on the basis of DNA extracted from soil and faecal samples, and showing the mutual interactions of particular network chains. These mutual relations in the rapidly changing Arctic ecosystem can be monitored relatively quickly and simply using metagenomics or eDNA techniques.

How the food web ecology in remote, pristine ecosystems can be affected was exemplified by the introduction of the Arctic fox in the Aleutian archipelago. Croll et al. (2005) showed that the decline in seabird densities caused by the introduction of Arctic foxes could influence the whole island environment. Based on $\delta^{15}\text{N}$ measurements from soil, plants and animal tissues, they showed that fox-free islands inhabited by large seabird populations were strongly subsidized by marigenic nutrients, which are the flywheel of the whole island ecosystem. Islands where the fox had been introduced, as a result of which the seabird populations became depleted, had a reduced nitrogen input from the sea to the land. Based on that example, it is easy to see how a food web evolves when one link in the chain is modified. In this specific case, introduction of the fox transformed the islands from grasslands to maritime tundra (Croll et al. 2005).

Environmental DNA

The focus on environmental DNA (eDNA) has led to a new approach to monitoring species in different environments. This technique is based on the fact that all animals leave DNA behind. By taking field samples and analysing them for eDNA, it is possible to

demonstrate the presence of a species without observing or capturing individuals (Valentini et al. 2009). Moreover, environmental DNA methods have enormous potential for the non-invasive detection, i.e. the identification, spread and abundance, population size estimates or conservation of highly sensitive target species. Environmental DNA also represents an important source of information on the present and past biodiversity of the flora and fauna (Mächler et al. 2013, Thomsen and Willerslev 2015).

The robustness of eDNA analysis has its potential problems, especially in case studies that do not aim to analyse the macrofauna or macroflora present in the environment. Since the analysis of such samples as faeces, plant or animal remains, or live microbial mats provides information that is relatively easy to interpret and is quite up-to-date, the results of deep soil analyses, for example, may be problematic. The reason for this is that the presence of DNA in the environment does not necessarily mean that the organism from which the DNA originated is currently present in this environment. Even in a warm climate, DNA degradation or the decay of the organisms may be not fast enough to yield the latest data about the content of live organisms or microorganisms in the sample (Liu et al. 2015). As the average temperature falls, the process also slows down (Kim and Wuertz 2015); in the Arctic the process may be extremely slow. Permafrost can preserve genetic traces of organisms that have been extinct for millennia. Suffice it to mention that it has been possible to sequence mammoths, which have been extinct for a few thousand years (Palkopoulou et al. 2015). One may anticipate similar problems with other organisms and microorganisms. This always has to be taken into consideration, so the results obtained from DNA analysis should always be treated with the utmost caution. Hence, eDNA analysis cannot be used as a stand-alone method but should always be supplemented by, or supplement, a comprehensive environmental study.

Another aspect of eDNA analysis is species identification, especially in the microbial world. Next Generation Sequencing enables thousands of 16S rRNA sequences to be obtained

and analysed relatively easily. These regions are typically sequenced using only a single pass, which can result in at least one sequencing error; in consequence, obtaining 100% identical sequences, even in such a highly conservative region, will be hard. This prevents us from using the biological meaning of 'species'. This approach requires some degree of sequence divergence (95%, 97% or 99% sequence similarity cut offs are typically allowed) and results in clustering into nearly-identical tags, referred to as Operational Taxonomic Units (OTU; clustering sequences based upon similarity) (Morgan and Huttenhower 2012, Nguyen et al. 2016). The sequences are stored and can be matched against several databases, such as GreenGenes (DeSantis et al. 2006), the Ribosomal Database Project (Cole et al. 2009) and Silva (Pruesse et al. 2007). It should also be borne in mind that the sequences in some databases are not accurate, neither is there any consensus quantitative definition of genus or species based on 16S rRNA gene sequence data. Consequently, as taxonomic assignments may depend on the reference database used, their results may change accordingly, and significantly so (Hiraoka et al. 2016). At present, identification to lower taxonomic levels, particularly to bacterial species, is seldom possible. The precision of this type of bacterial community member identification will increase over time, as databases are updated and as more sequences become associated with lower levels of the taxonomic range of microbes (Morgan and Huttenhower 2012, Nguyen et al. 2016). Furthermore, horizontal gene transfer (HGT) between distinct taxa may lead to incorrect taxonomic assignments (Pignatelli et al. 2008). When comparing a microbial community with an environment, it is important to remember that DNA isolation impacts the structure of the bacterial community. A particular isolation method may be more effective with some taxa but less so with others (Krsek and Wellington 1999, Carrigg et al. 2007).

Despite these problems, eDNA can provide a lot of valuable information based on basic molecular techniques. Different tools employing microsatellite DNA, as well as the

mtDNA markers used in forensic genetics, can be used to identify animals based on their traces. Faecal DNA analysis may be potentially valuable for the conservation and management of endangered but rarely encountered species, as molecular techniques are easier to carry out and faster than traditional approaches based on observation, behaviour and trapping.

Glacial ice and permafrost are also a source of ancient DNA (aDNA). Studies of aDNA gives us information not only about microorganisms but also about extinct animals, plants and other organisms (Pedersen et al. 2016). The main problems faced by aDNA investigation are low yield, contamination by modern DNA and degradation (Mitchell et al. 2005). Using the right procedure and methods, however, and following strict criteria for authentication lower the risk of contamination and false results (Hebsgaard et al. 2005, Thomas et al. 2005). Studies of aDNA isolated from permafrost across the whole Arctic have given us the most extensive historical records of vegetation changes over the past 50 kyr (Willerslev et al. 2014). Different microbial community structures were found in two different permafrost samples of a similar age (Krivushin et al. 2015). It was shown, for example, that bacterial communities from different layers of the Canadian permafrost exhibit diverse resistance mechanisms against aminoglycoside, β -lactam and tetracycline antibiotics (Perron et al. 2015).

Metagenomics in environmental research

Metagenomics is the study of genetic material recovered from microorganisms that cannot be cultured with the use of currently known molecular methods, directly from their natural environment (Handelsman et al. 1998, Daniel 2005, Schloss and Handelsman 2005, Sabree et al. 2009, Thomas et al. 2012). Although metagenomics is still quite a new branch of science and a radical new way of doing microbiology, it has already delivered plenty of

information about uncultured microorganisms. This approach has revealed an enormous diversity of unknown and uncharacterized biochemical pathways, providing biological insights into complex microbial communities (Handelsman et al. 1998, Tyson et al. 2004, Venter et al. 2004). All metagenomics studies have a similar workflow and take the same first step: DNA is extracted directly from all the microbes living in a particular environment (Handelsman et al. 2007). DNA from microorganisms can be extracted from various environments, such as soil, sediments, water, sludge, oral cavity, faeces and skin (e.g. Saano et al. 1995, Snaidr et al. 1997, Daniel 2005, Wexler et al. 2005, Tekere et al. 2011). In the next step, DNA can be sequenced, then directly analysed or cloned into a form maintainable in a laboratory, creating a library that contains the genomes of all the microbes found in that environment (Handelsman et al. 2007).

Recent advances in high-throughput sequencing technologies provide access to the culture-free characterization of microbial community structures in a variety of environments, including soil and faeces. Next Generation Sequencing (NGS) makes it easier and faster to analyse the structure of bacterial communities without the need to clone DNA sequences into a vector (Handelsman et al. 2007). Moreover, metagenomic approaches allow an earlier unidentified microbial DNA to be obtained that can lead to the discovery of new genes encoding proteins with a desired function, for example, resistance to antibiotics, or involved in pollutant degradation (Riesenfeld et al. 2004, Daniel 2005, Zhang and Bennet 2005, Handelsman et al. 2007). Because of our inability to culture as many as 99% of bacteria using traditional microbiological methods, the direct extraction of microbial DNA from environmental samples has become the method of choice in molecular biology and biotechnology research when it comes, e.g. to estimating microbial biodiversity, searching for genes with a desired function or recognising the relationship between the structure of the genome and the part it plays in a specific environment (Schmidt et al. 1991, Zhou et al. 1996,

Riesenfeld et al. 2004, Daniel 2005, Deja-Sikora et al. 2007, Neelakanta and Sultana 2013). Furthermore, NGS provides an opportunity to analyse low abundance microbial components that contribute to less than 1% of the total population (Lee et al. 2012).

In microbial ecology, metagenomics is used for describing the taxonomic distribution of microbial community members. In this context, NGS sequence analysis of the 16S rRNA gene becomes the ideal tool for obtaining taxonomic information, often considered to be the ‘gold standard’ as regards bacterial identification; in consequence, the gene sequence has been targeted in countless studies (Rosselli et al. 2016). The 16S rRNA gene is often used in studies of bacterial evolution and ecology, including the determination of phylogenetic relationships among taxa and the exploration of bacterial diversity for several reasons. The most important one is the fact that the gene is universally distributed, which allows one to analyse phylogenetic relationships among distant taxa. Being functionally indispensable, this gene is also highly conserved. Nevertheless, the 16S rRNA gene is also variable in certain regions, which allows sufficient diversification and makes it a tool well-suited for classification (Head et al. 1998, Větrovský and Baldrian 2013).

Regardless of above, there are several aspects limiting the interpretation of 16S rRNA-derived results. The most important one is the fact that its copy numbers per genome vary from 1 to 15 or more (Klappenbach et al. 2001). Nowadays, moreover, most analyses of bacterial community structures rely on OTU analysis and the construction of similarity clusters of 16S rRNA gene PCR amplicons (Morgan and Huttenhower 2012, Nguyen et al. 2016). Consequently, both multiplicity and variability present problems for the assessment of bacterial diversity and community structure, i.e. the relative abundance of individual taxa. Another drawback of 16S rRNA gene analysis is that the presence of DNA may not necessary mean that the organism detected is alive and still present in the environment, especially when

one considers the Arctic environment, where low temperatures can preserve DNA for a long time and the microbial structure obtained may not represent the current microbial community.

A possible solution to this would be to focus on RNA rather than on DNA, as rRNA itself can yield useful information about which community members are currently active (Kamke et al. 2010, Wemheuer et al. 2012). Unfortunately, there are also problems with this approach, as total RNA has to be extracted from an environmental sample, a quite problematic step in itself, and subsequently converted to cDNA. The library for the NGS analysis of 16S rRNA amplicons is generated from cDNA, and still dependent on a PCR process, as cDNAs are amplified by universal primers, as is the case in 16S rRNA gene analysis (Kamke et al. 2010, Wemheuer et al. 2012, Rosselli et al. 2016). Furthermore, Rosselli et al. (2016) propose the direct sequencing of 16S ribosomal RNA without any primer- or PCR-dependent step in order to overcome the above-mentioned drawbacks. Direct rRNA-seq is based not only on taxon abundance, but also on physiological activity and could be used as a methodology complementary to those mentioned above in microbial community studies (Rosselli et al. 2016).

To date, a series of metagenomics studies has revealed a high diversity of microbes in different niches and, in consequence, improved our understanding of new taxa, their biogeographical distribution and the association of specific microbial groups with geochemical factors (Guan et al. 2013). Moreover, metagenomics approaches allow one to explore the distribution of microbial populations, to compare microbial communities in different environments influenced by various natural or anthropogenic factors, and to monitor the effect of ongoing environmental changes on microbial communities (Neelakanta and Sultana 2013). There are several studies concerning the high Arctic involving metagenomics approaches. Varin et al. (2012) analysed the metagenomes of cyanobacterial mats from Arctic and Antarctic ice shelves using high-throughput pyrosequencing. Their study highlights the

presence of diverse mechanisms of adaptation to cold and other stresses in polar mats, which is connected with the representation of major bacterial groups. Lay et al. (2013) provided insights into the metabolic potential and the active microbial populations that exist in the hypersaline cryoenvironment (Canadian high Arctic) by means of metagenomic analyses of the microbial community and pyrosequencing analyses of the cDNA of its 16S rRNA genes. Pawłowska et al. (2014) used metagenomics approaches to compare the diversity of foraminifera in sediments from the Hornsund fjord (Svalbard) with the diversity of aDNA sequenced from subsurface sediment samples. Also in Svalbard, in the high Arctic, Larose et al. (2013) investigated changes in the microbial community associated with seasonal atmospheric Hg deposits by using metagenomic approaches to quantify mercury resistance genes in snow samples.

Molecular identification based on environmental samples

The DNA-based approach for studying the relationships between individuals, populations and species has become an important aspect of molecular ecology and in consequence of conservation genetics and systematics (Hellborg and Ellegren 2002). Many molecular tools have been used to perform molecular identification of both species and individuals, including sex identification, also in the context of their health conditions and food intake. All that information can be a valuable source of data about animals inhabiting a particular area, especially when a small number of individuals live in a large area and their presence is recorded only on the basis of faecal residues. The Y chromosome-linked approach, together with other molecular markers like mtDNA or microsatellite DNA, can also be used for identifying species in the context of animal relatedness in the population, and their relationships within a given environment in the context of its ecological aspects.

Ruokonen et al. (2005) used mtDNA analyses to investigate population structure and phylogeography of the pink-footed goose (*Anser brachyrhynchus*) from breeding areas in

Greenland, Iceland and Svalbard. They indicated that populations of pink-footed goose separated recently (less than 10 000 years ago) and that present breeding areas were colonized from one refugial population. Mitochondrial DNA isolated from blood, muscle and feathers collected in breeding areas also supported the concept of three species: the pink-footed goose, breeding in Greenland, Iceland and Svalbard, Middendorff's goose *Anser middendorffi* breeding in the eastern taiga zone, and the bean goose *Anser fabalis*, breeding in the western parts of the taiga zone and in the tundra zone of the Palearctic (Ruokonen et al. 2008).

Investigation of mtDNA collected from tissue, muscle and skin showed the evolutionary distinction of north-west Greenland walruses from populations in east Greenland, Svalbard and Franz Josef Land. Moreover, microsatellite variation grouped Atlantic walruses into three populations: one in north-west Greenland, another in east Greenland and a common Franz Joseph-Svalbard population (Andersen et al. 1998).

The origin of the three small-bodied, high-Arctic subspecies of reindeer were investigated by sequencing the control region of mtDNA from tissue, blood, skin and bone. *Rangifer tarandus pearyi* from the Canadian Archipelago and *Rangifer tarandus groenlandicus* from east Greenland (extinct since 1900 AD) were closely related and probably evolved in high-Arctic North America, whereas *Rangifer tarandus platyrhynchus* from Svalbard evolved from Eurasian large-bodied reindeer (Gravlund et al. 1998). In addition, data from 14 microsatellites were used to characterize the levels of genetic diversity of Svalbard reindeer from two adjacent areas on Nordenskjöldland, Spitsbergen. That study acknowledged the fact that Svalbard reindeer appear to conform to the paradigm of island populations made genetically depauperate by genetic drift (Côté et al. 2002). The Y chromosome-linked approach has already been shown to be useful for sex identification based on Svalbard reindeer faecal samples from the northern part of the Hornsund fjord (Zielińska et al. 2016b).

This approach, namely the analysis of eDNA from Arctic samples, is also widely used in the identification not only of microbes and fungi (Maccario et al. 2014), but also of potential metabolic responses of microbial communities to environmental stresses (Varin et al. 2012) and their contribution to greenhouse gas emissions (Yergeau et al. 2010). Metagenomics studies of eDNA have been successful in the identification and estimation of plant taxonomic diversity across many climate zones (Yoccoz et al. 2012) and microbial and fungal species present in Arctic snow (Maccario et al. 2014). Moreover, when combined with pollen and macrofossils, the method has proven its worth in the palaeovegetational reconstruction of vegetational changes of Siberian regions from material deposited in permafrost (Jørgsen et al. 2012). Large-scale analysis of eDNA has yielded insight into plant and nematode growth and the diet of herbivorous megafaunal mammals over a time span of 50 000 years in the Arctic (Willerslev et al. 2014). Analysis of DNA from glacier ice enabled multiple taxa of fungi, plants, algae and protists to be identified, a result that could not have been obtained using morphological identification alone (Willerslev et al. 1999). Analysis of deep ice cores obtained from Greenland have revealed a rich source of DNA showing a diverse array of coniferous trees and insects within the past million years of the history of the sampled area (Willerslev et al. 2007).

Faeces permit non-invasive sampling

Over the years, advances in molecular techniques have enabled microbial animal and plant identification at both species and individual levels by DNA analysis based on non-invasive material like faeces, urine, shed hair or feathers (e.g. Taberlet et al. 1997, Kohn et al. 1999, Ernest et al. 2000, Flagstad et al. 2004), which are valuable sources of environmental DNA. In ruminants, samples for microbial analysis are usually collected from the rumen (Makkar and McSweeney 2005, Sundset et al. 2007, Lee et al. 2012, Glad et al. 2014).

399 However, this kind of sampling strategy, though reasonable, can only be performed on a dead
400 animal, otherwise it causes the animal serious stress. Moreover, such sampling methods
401 interfere with the natural animal environment, especially in the case of wild populations with
402 limited or no previous exposure to humans (Zielińska et al. 2016a). In such situations, and
403 also in case of other animal species, testing of faecal samples as a non-invasive sampling
404 strategy can be a good alternative, especially when the fieldwork takes place in wild,
405 uninhabited areas. Faecal samples can provide much valuable information about the symbiotic
406 microorganisms, bacteria, archaea or fungi associated with a particular species or
407 environment.

408 Faecal samples have often been used in traditional cultivation methods for screening
409 bacteria of potentially pathogenic significance to humans. The barnacle goose *Branta*
410 *leucopsis*, a migratory bird, was tested as a potential carrier of virulent strains of *Escherichia*
411 *coli* into the Arctic environment (Hatha et al. 2013). With the use of traditional cultivation
412 methods combined with molecular techniques, cloacal swabs and faeces samples of wild birds
413 from Arctic Svalbard (Norway) were analysed for *Escherichia coli* and *Enterobacter cloacae*
414 isolates, resistant to cephalosporins and fluoroquinolones, and for *Salmonella* isolates. As a
415 result, a novel variant of the *ampC* beta-lactamase gene (blaACT-23) was revealed (Literak et
416 al. 2014). Also, molecular techniques revealed shiga toxin genes from pathogenic *E. coli* in
417 Svalbard reindeer faeces (Zielińska et al. 2016b).

418 A study of pathogenic yeasts in cloacal swabs from the little auk (*Alle alle*) suggested
419 that this seabird is a random carrier of such yeasts (Dynowska et al. 2013). Nonetheless,
420 owing to the specific composition of faeces, the range of applicability of certain analytical
421 techniques like metagenomic analysis is limited. In our experience, extraction of good quality
422 DNA can be very difficult, and is even impossible at present for some bird species: this is

because of the strong enzymatic inhibitors present in birds' faecal, and sometimes because the sample is too small.

Faeces can also be a valuable source of information about microorganism composition. Metagenomic approaches were used to investigate bacterial diversity in polar bear faeces (*Ursus maritimus*) from Svalbard (Glad et al. 2010). With the aid of the 16S rRNA gene clone library, they studied the microbial structure of the bear's gut, hitherto little known. They also investigated the bla_{TEM} alleles, encoding ampicillin resistance, as a potential natural reservoir of those clinically important genes. In another study, bacterial population structure was investigated using Next Generation Sequencing (NGS) 16S rDNA analysis of faecal samples from the endemic population of Svalbard reindeer in the northern part of the Hornsund fjord (Zielińska et al. 2016a).

DNA extracted from faeces can be used as a comprehensive and non-invasive tool in order to establish the diet of the animals under scrutiny. DNA barcoding (species identification using a standardized DNA sequence), together with the availability of DNA sequencing techniques, presents new opportunities in animal diet analysis. DNA fragments, often degraded and shorter than 100–150 bp, are easily found in faeces. By using universal primers for amplifying very short but informative DNA fragments, one can identify not only food components of animal origin but also the plant taxa that had been consumed. Even so, identification of prey using this method can be quite challenging and is not always precise. Such analyses, e.g. mtDNA analysis of prey items employed to establish animal feeding ecology, have been useful for research into many bird species, like Adelie penguins (*Pygoscelis adeliae*) (Jarman et al. 2002), Macaroni penguins (*Eudyptes chrysolophus*) and little penguins (*Eudyptula minor*) (Deagle et al. 2007, 2010).

Joo et al. (2014) applied length-heterogeneity analysis by PCR (LH-PCR) based on length differences of internal transcribed spacer (ITS) regions of ribosomal RNA (rRNA) to

study foraging preference on Svalbard from Ny-Alesund, north-west Spitsbergen, on the basis of faecal samples. They demonstrated that this approach could solve a number of difficulties typically encountered in conventional sampling methods. In order to do so, they created a length-heterogeneity (LH) database from samples of potential food sources of Svalbard reindeer and of their faeces. Detailed and accurate information on foraging preferences in accordance with seasonal and regional characteristics can help to understand the energy requirements of Svalbard reindeer, which are related to the quality and quantity of the food they ingest. These authors suggest that LH-PCR for faecal dietary analysis would be an efficient, non-invasive monitoring tool for characterizing the foraging strategy of Svalbard reindeer. This approach could also be used for other Arctic animals in order to obtain comprehensive dietary information based only on field-collected faecal samples. Such samples can thus be treated as valuable sources of information, delivering comprehensive data about the identity of a species as well as its genetic history, microbiome (also specific genes) and diet.

Soil microbiology

Soil is often described as the most challenging of all natural environments with regard to DNA extraction and analysis, mostly because of the diversity of species present in it, especially microorganisms, and the variety of enzymatic inhibitors, like humic acids or heavy metals. Soil can also contain mineral particles of different size or origin, as well as organic compounds that can be at various stages of decomposition (Daniel 2005). In the soil environment, microorganisms (such as bacteria and fungi) play an extremely important role in decomposing dead organic matter and in the nutrient cycle generally. Any adverse changes to soil parameters, such as pollution, pH, temperature and biomass, can disrupt the nutrient

cycle, which may have an impact on the functioning of the whole ecosystem (Stefanowicz et al. 2009).

Studying the microbial communities of the Arctic can be a valuable source of information about how that ecosystem functions. Many studies have focused on determining the microbial community structure and its relationship with changing soil properties, not only in the Arctic (Campbell et al. 2010, Ramirez et al. 2010, Kim et al. 2014). Owing to the harsh environmental conditions, microbial communities in the Arctic soil were expected to have a poor diversity of bacterial species, but in fact they are as diverse as those found in other biomes (Koyama et al. 2014). Moreover, Chu et al. (2010) demonstrated that the bacterial communities of Arctic soils have similar levels of variability, richness and phylogenetic diversity as soils from a wide range of lower latitudes, which suggests a common diversity of structure within soil bacterial communities around the globe. These authors concluded that Arctic soil bacterial communities are strongly influenced by local environmental factors, especially those associated with soil acidity, similar to what was observed for the fungal community composition in this high Arctic region (Zhang et al. 2016). In addition, cyanobacteria communities depend not only on soil crust development but on pH, ammonium and organic carbon concentrations: a higher pH and a lower concentration of total organic carbon resulted in a lower cyanobacteria richness (Pushkareva et al. 2015).

Mackelprang et al. (2016) indicated that the striking differences in bacterial composition and diversity among different permafrost locations might be due to stark variations in permafrost chemistry, mineralogy and ice content. Furthermore, Allison and Martiny (2008) imply that changes in microbial composition are often associated with changes in ecosystem process rates. Thus, changes in microbial communities resulting from disturbances may directly affect ecosystem processes. Establishing these relationships can determine how easily the microbial community composition can be used to predict the

responses of the ecosystem to global change. The same appears to apply to root-associated fungal communities, where no relationships between them and above-ground Arctic vegetation were found (Mundra et al. 2015). The richness of root-associated fungal increased with host root weight and length (Mundra et al. 2015). The fungal community structure depended on the pH, C:N ratio and available phosphorus (Fujimura and Egger 2011).

Microorganisms mediate crucial biogeochemical cycles of nitrogen and carbon in cold environments (Makhalanyane et al. 2016). Thus, knowledge of soil microbial community structures and functions is important for predicting the response of Arctic terrestrial ecosystems to climate changes. It has been shown that changes in Arctic peat soil microbiota were temperature-dependent. Above 7°C a shift was observed where *Firmicutes* were replaced by *Bacteroidetes*. This was evidence for the rapid adaptation of microbial communities to increasing temperatures and their influence on CH₄ production (Tviet et al. 2015). In Alaska, too, the microbial communities of the permafrost respond to thawing by rapidly stimulating enzymatic activity, which decomposes organic matter in the soil (Mackelprang et al. 2011, Coolen and Orsi, 2015).

Nonetheless, studies on Spitsbergen have shown no significant differences in the high taxonomic ranks of bacterial structure communities between areas intensively fertilized by the little auk colony, characterized by high levels of nutrients and organic matter biomasses, and the control area (Zielińska et al. 2016c). In this case, different physicochemical features of the soil had no substantial impact on the general bacterial community structure, at least at higher taxonomic ranks.

Conclusions

Owing to the harsh climate, presence of permafrost, short growing season, strong seasonality and rapid climate changes, studying Arctic ecosystems is a high-priority issue

(Gough et al. 2016, Makhalanyane et al. 2016). Their current state and functioning need to be known and understood in order to monitor changes and their influence on particular aspects of the ecosystem. Ecological relationships and climate-induced changes in ecosystems can be monitored faster and more easily using molecular approaches such as metagenomics or environmental DNA techniques. Molecular techniques enable species to be identified and even whole population structures to be established on the basis of environmental DNA samples. By combining molecular and ecological techniques one can acquire detailed information about plants and the individual features of animals, including their diet and symbiotic microorganisms. Even though there are potential problems with the analysis of DNA from environmental samples, molecular approaches can be a valuable supplementary source of information about the structure and functioning of the Arctic ecosystem in the course of transformation.

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Figures

Figure 1. Example of an ecological network. Relationships between particular chains based on total DNA (microbial DNA, mtDNA and chromosomal DNA) extracted from soil and faecal samples.

Figure 1. Example of an ecological network.
Relationships between particular
396x280mm (72 x 72 DPI)