Abstract

Tardigrades are common in most terrestrial habitats. Although little is known about their ecological preferences, previous studies have found that tardigrade diversity and community composition are significantly affected by various ecological variables. In this study, we associated georeferenced tardigrade species records with climatic variables, forest type, and substrate type to explore tardigrade diversity, and species communities to see if they can be associated with ecological characteristics of Norwegian forests.

A total of 17474 specimens were identified, encompassing in total 131 species (including species putatively new to science) from 321 samples of leaf litter bryophytes and lichens. Bryophytes and lichens of each sample were classified according to the main species, life form, and growth form, and associated with tardigrade species and sample metadata. Tardigrade species richness increased with precipitation, but did not change with temperature or precipitation seasonality. Species richness was related to forest type, with greatest values in mixed oak and birch forests, and lowest in lime forests. When correcting for sample size and multiple pairwise comparisons, there were no significant differences in species richness between substrates. Tardigrade community composition varied between substrate types and to a lesser degree between forest types, but not with climatic variables.

INTRODUCTION

Tardigrada (water bears) is a phylum of microscopic animals inhabiting the great majority of ecosystems throughout the world. They are aquatic animals in the sense that they require being surrounded by water for oxygen uptake and support of the hydrostatic skeleton when there are in active life stage. Many species are nevertheless adapted to (limno-)terrestrial habitats that dry out regularly by an ability to survive complete desiccation and freezing (e.g. Guidetti *et al.*, 2011; Kaczmarek *et al.*, 2019; Møbjerg *et al.*, 2011; Wełnicz *et al.*, 2011). Limnoterrestrial tardigrades are found mainly in mosses, lichens and soil (e.g. turf and leaf litter) habitats, all of which are common in boreal forests (Nelson *et al.*, 2015). Tardigrades are herbivorous, predators, and bacteriovorous. They can be important for the decomposition of leaf litter and soil formation and high densities have been reported from young soils (Hohberg, 2006; Hohberg *et al.*, 2011). Tardigrades are also a food for many other invertebrates (Nelson *et al.*, 2015), which is important in young soils where the food sources generally are very poor. Thus, tardigrade communities may play an important role in the regeneration of forests.

Although common in most habitats, few studies have focused specifically on tardigrade diversity in forests. However, those that have investigated forest ecosystems document high densities and species diversity (Dastych, 1980; Degma *et al.*, 2005; Guidetti *et al.*, 2021; Guidetti *et al.*, 1999; Harada & Ito, 2006; Ito, 1999; Ito & Abe, 2001; Jönsson, 2003; Kaczmarek *et al.*, 2011b). In leaf litter, densities up to 90 000 specimens/m^2 and 12 species per sample have been recorded (e.g. Guidetti *et al.*, 1999; Guil & Sanchez-Moreno, 2013). The number of tardigrade species inhabiting forests in Norway is unknown, but 61 of the species so far recorded in Norway are found in different types of forest habitats and an additional 25 are found in limnic environments in forests (Meier, 2017).

Although little is known about the ecological preferences of many species, several studies found that ecological variables significantly affected tardigrade diversity and community composition (e.g., Bingemer *et al.*, 2020; Dastych, 1988; Guidetti *et al.*, 1999; Guil & Sanchez-Moreno, 2013; Kaczmarek *et al.*, 2011b; Nelson *et al.*, 2020; Vecchi *et al.*, 2021;

Zawierucha *et al.*, 2015; Zawierucha *et al.*, 2017; Zawierucha *et al.*, 2016). Thus, comprehensive faunistic research associated with habitat and substrate types is needed to evaluate the ecological factors affecting tardigrade species communities and the role which tardigrades might play in forest ecosystems. Recent studies on tardigrade biodiversity were mainly focused on the discovery and description of new species and the composition of entire tardigrade communities inhabiting different substrates and/or nature types remains mostly unknown. Extensive ecological-faunistic studies and increased knowledge of the distributions, auto- and synecology, and adaptation are needed to better understand the community structure, habitat preferences and the role tardigrades might play in various ecosystems.

The first tardigrade species was formally described in 1834 (Schultze, 1834) and the first report on tardigrades from Norwegian territory appeared almost thirty years later in a study on terrestrial fauna of Spitsbergen (Göes, 1862). It was only at the beginning of the 20th century that tardigrades were discovered in mainland Norway (Richters, 1903). Subsequently, very few studies on tardigrade diversity have been performed in continental Norway (e.g. Durante Pasa & Maucci, 1975; Durante Pasa & Maucci, 1979; Mihelčič, 1971; Mihelčič, 1971/72; Richters, 1904) with only two of them extensive (Durante Pasa & Maucci, 1979; Mihelčič, 1971). The most recent checklist of Norwegian tardigrades includes a total of 146 species from Norway, including 102 species recorded from mainland Norway and 97 from the Svalbard archipelago (Meier, 2017). However, the list includes several species with unclear taxonomic status, like some members of the genus *Mesobiotus* Vecchi *et al.*, 2016 or species in the *Macrobiotus hufelandi* group. Additionally, tardigrade species belonging to complexes like the *Hypsibius convergens-dujardini* group, *Mesobiotus furciger* group, *Mesobiotus harmsworthi* group, *Ramazzottius oberhaeuseri* group, *Macrobiotus pallarii* group, *Diphascon pingue* group or the genera *Milnesium, Minibiotus, Paramacrobiotus* and *Pseudechniscus* are tricky to identify and these taxa likely hide cryptic species and species new to science (e.g. Cesari *et al.*, 2019; Fontoura & Pilato, 2007; Gąsiorek *et al.*, 2018; Grobys *et al.*, 2020; Guidetti *et al.*, 2019; Guil *et al.*, 2022; Kaczmarek *et al.*, 2020a;

Kaczmarek *et al.*, 2022; Morek *et al.*, 2021; Roszkowska *et al.*, 2020; Stec *et al.*, 2020; Stec *et al.*, 2018; Stec *et al.*, 2021). On top of this, current species records from Norway are the result of patchy, uneven sampling. Thus, a higher number of species records for Norway is expected. Moreover, since current records usually are in very low numbers per sample, background data to investigate habitat preferences is not available and extensive comparisons of tardigrade diversity and community composition in different forest types has yet to be explored.

The forests in Norway are characterised by a number of environmental variables and are divided into no less than 20 different ecosystem types using the EcoSyst framework in "Nature in Norway" (Halvorsen *et al.*, 2020). The localities selected for our study represent a subset of seven forest ecosystems dominated by different types of main vegetation: Norway spruce-dominated bilberry forest, calcareous lime forest, mixed deciduous forest with alder, mixed oak forest, birch forest, broad-leaved deciduous forest and Norway spruce-dominated small fern forest. In concert, these represent a range of climate- and productivity variables in the southern half of Norway (Gjerde *et al.*, 2005), and have been used extensively in diversity assessments and surveillance programs (e.g. Brandrud *et al.*, 2011; Gjerde & Baumann, 2002). Bryophytes and lichens are very moist substrates in most of the studied forests and stay moist even during periods with little rain as the regional climates are mainly oceanic. Hence, they make up important habitats for a wide variety of invertebrates, from protozoans to insects (Longton, 1992). Bryophytes and lichens are small-sized organisms, but the surface area of the many leaves and lobes is huge, in particular for microbiota (Glime, 2017), offering shelter, food and a place for reproduction. Especially wefts of pleurocarpous mosses, large cushions of acrocarpous mosses and large foliose lichens make complex structures housing large amounts of algae, fungi, cyanobacteria etc. for tardigrades to feed on. Some microbiotas, including some tardigrades, feed on lichens and bryophytes directly (Longton, 1992) whereas others feed on for instance algae and bacteria.

In this study we investigated tardigrade diversity at community-level across Norwegian forest types and explore if they are associated with ecological variables including climate, forest type and substrate type.

MATERIAL AND METHODS

A total of 321 samples of bryophytes (mosses and liverworts), lichens and leaf litter from 12 forests in central and southern Norway in the summers of 2017 and 2018 were collected and analysed (Fig. 1, Table 1, Supporting Table S1). The material included 112 samples from bryophytes (mosses and liverworts), 110 from lichens, and 99 from leaf litter. In some forests, we distinguished between sub-localities if these had substantially different main vegetation.

Samples were taken by placing a metal ring delimiting approximately 10 cm^2 of the substrate, collecting all organic substrate from inside the ring, and placing these in separate, labelled, sterile whirl-pak bags. The selected substrates were bryophytes and lichens from trees, ground, rocks, and boulders, and leaf litter. Except for juveniles, bryophytes (mosses and liverworts) and lichens were identified to species level using suitable literature (Hallingsbäck, 2016; Holien & Tønsberg, 2008). Samples were kept refrigerated or frozen until dried at 35 °C in a ventilated drying oven for 2-3 days. The samples were subdivided among three laboratories (i.e. EvoZoo lab led by R. Guidetti, Kaczmarek's lab, and Meier's lab) for tardigrade extraction and analyses. Sub-samples roughly equal to 25% of the original samples were then weighed and immersed in tap water in a large beaker for 10 min, while stirring vigorously for 1 min. Large pieces of the substrate (e.g. plant, lichen or litter material) were removed by hand and the remaining water mixture was sieved through a sieving stack where the bottom sieve had 0.037 mm mesh. The sieve stack was washed thoroughly and the remains in the bottom sieve examined in a stereomicroscope to extract animals, exuviae and eggs. If no tardigrade specimens were found within 30 min, the sample was discarded. Sieves were thoroughly cleaned between each sample. All specimens (adults, immatures, exuviae, and eggs) in the sample were retrieved and included in the downstream analyses

after slide-mounting in Hoyer's or Faure's solution for identification. Slides were sealed with nail polish or 'International Toplac' boat paint to avoid desiccation of the mounting medium. Reference specimens are deposited in the NTNU University Museum (NTNU-VM), Norwegian University of Science and Technology (Norway), in the Adam Mickiewicz University in Poznań (Poland), and in the Bertolani collection of the University of Modena e Reggio Emilia (UNIMORE) (Italy).

Tardigrades were identified using available literature and reference specimens in the collections of Bertolani (UNIMORE), Kaczmarek lab and Meier. The most relevant literature used were: Fontoura & Pilato (2007), Kaczmarek *et al.* (2020b), Kaczmarek *et al.* (2017), Kaczmarek & Michalczyk (2017), Kaczmarek *et al.* (2018), Michalczyk & Kaczmarek (2010), Morek *et al.* (2016), Pilato & Binda (2010), Ramazzotti & Maucci (1983), Roszkowska *et al.* (2020), Tumanov (2020).

To ensure conformity in species-level identifications between identifiers, three workshops were held where morphological characteristics of each taxon were discussed and a common nomenclature for interim names was agreed upon. Since cryptic species are common in some genera and species groups, and many species are identifiable only if animals and eggs are available (Guidetti *et al.*, 2019; Kaczmarek *et al.*, 2017; Kaczmarek & Michalczyk, 2017; Tumanov, 2020), two datasets were used in the statistical analyses. One dataset (Dataset 1) considered all the identified taxa, while for the other (Dataset 2) a conservative approach was taken: specimens identified to species belonging to *Macrobiotus* gr. *hufelandi*, *Paramacrobiotus* gr. *richtersi* or the genus *Mesobiotus* were considered at the species groups or genus group level, respectively. Thus, identifications across samples were at a comparable level even if eggs or other characters needed for species-level identifications were unavailable. After this generalisation of the taxonomy, the two datasets were used in the statistical analyses: Dataset 1 with 131 taxa that were represented in the **diversity analyses** (Supporting Table S1), Dataset 2 with 125 taxa that were represented in the **community composition analyses** (Supporting Table S1). Species richness (r_s = -0.01; Spearman´s rank correlation) and Shannon indices (r_s = 0.04) were not correlated with

sample mass and therefore samples were not normalised by weight in the downstream analyses.

Diversity analysis was undertaken using both species richness (or taxonomic richness - see previous paragraph) and Shannon diversity index (accounting for both richness and evenness of species abundances). We used linear models to test the hypotheses that the diversity of tardigrades varied with (i) climate, (ii) forest type (e.g. spruce, broadleaf, etc.) and (iii) substrate type. Substrate type was classified according to the growth form of the bryophyte, liverwort or lichens in the substrate (i.e. fruticose, foliose or crustose lichens, thallose or leafy liverworts, pleurocarpous or acrocarpous mosses), or as leaf litter; these are shown in Supporting Table S1. Pairwise comparisons between forest types or substrate types were undertaken using the Tukey method to correct for multiple comparisons, using the emmeans package (Lenth, 2022). Our evaluations of statistical significance are based on the Tukey-adjusted p-values, but in Supporting Table S3 we also provide the uncorrected p-values since the use of alpha level corrections in multiple tests has been subject to much debate (e.g., Armstrong, 2014; Rubin, 2017). Three bioclimatic variables were used: mean temperature of the warmest quarter (referred to as summer temperature herein), annual precipitation and precipitation seasonality. These represent the three main axes of bioclimatic variation in Norway (Speed & Austrheim, 2017). Elevation and latitude of a site were not used as they are collinear with temperature. The bioclimate data was downloaded from WorldClim2 (Fick & Hijmans, 2017). Backward model selection was undertaken from a full model including all variables (three bioclimate variables, forest type and substrate type), based on likelihood ratio tests, using an alpha of 0.05.

Compositional analyses were undertaken through distance-based redundancy analysis, based on the Bray-Curtis distance index. We first used an unconstrained ordination, and then fit convex hulls for each identifier to investigate potential identifier-biases in the identification of taxonomic units from samples. Next, we fitted a constrained ordination on the three bioclimate variables, forest type and substrate type (as outlined above). We used permutational analysis of variance to test whether tardigrade community composition (the

composition of tardigrade taxa within single samples) varied with these ecological variables (forest type, substrate type and the three climate variables) and we used analysis of multivariate homogeneity of group dispersion to test whether the variance (beta-diversity) between substrate and forest types was homogenous. All statistical analyses were undertaken in R 4.1.3 (R-Core-Team, 2022), using the vegan package (Oksanen *et al.*, 2022) to analyse diversity and composition.

RESULTS

General faunistic results

A total of 17474 specimens (including eggs) were found in the 321 analysed samples, encompassing in total 131 putative species or species groups from 37 genera (Supporting Table S1). Among these, 48 species were identified to species level, 14 species were uncertain assignments (denoted "cf.") and 11 taxa could only be assigned to genus or a species group name (Table 2). No less than 58 species were given interim names as they were morphologically distinct but could not be associated with a formally described taxon due to the lack of taxonomic revisions or because they are new to science.

Comparing the identifications of the specimens obtained in the three laboratories involved in the taxonomic work (i.e. Guidetti's Lab, Kaczmarek's Lab and Meier's Lab), the unconstrained dbRDA (i.e. PCoA) based on Bray-Curtis dissimilarity showed a close overlap in the taxa identifications, indicating no identifier bias in the identified samples and reliable taxonomic data (Supporting Fig. S1).

Biodiversity indices and environmental variables

We investigated if species richness and Shannon diversity index correlated with precipitation regime, precipitation seasonality, summer temperatures, forest types or substrate types. Precipitation regime and forest type in which the samples were collected explained more variance in diversity than substrate type. For species richness, a higher proportion of variance was explained by annual precipitation ($F_{1,270}$ = 33.6, P<0.001) and forest type ($F_{5,270}$

= 24.9, P<0.001) than by substrate type ($F_{7,270}$ = 2.3, P<0.05), but all three variables were significant predictors in the final model. For Shannon diversity, annual precipitation explained far more of the variance ($F_{1,277}$ = 25.3, P<0.001) than forest type ($F_{5,277}$ = 7.7, P<0.001), while substrate type had no significant effect and was not retained in the final model.

While both the species richness and Shannon diversity index of tardigrades varied with annual precipitation, neither diversity measure varied with summer temperature or precipitation seasonality (Fig. 2). Both species richness and the Shannon diversity index were higher in locations with higher annual precipitation. However, the increase was modest with approximately 3.6 (SE = 0.6, P<0.001) additional species per 1000 mm precipitation (Fig. 2). Shannon diversity increased by 0.60 units (SE = 0.01 P<0.001) per 1000 mm precipitation (Fig. 2).

The total number of species recorded in each substrate type varied across forest types (Table 3). Without correcting for sample size, the higher number of species were recorded in birch and lime forests and in foliose lichens, leaf litter and pleurocarpous moss, respectively (Table 3). Species richness of tardigrades also varied between both forest types and substrate types (Fig. 3a, Fig. 4a, Supporting Table S2). Species richness was greater in mixed oak (mean = 9.9 +/- 0.84) and birch forests (7.6 +/- 0.598), and lowest in lime forests (3.2 +/- 0.28; Fig. 3, Supporting Table S3). The species richness was significantly greater in mixed oak forests than in all other forest types with the exception of birch forest (P<0.001 for all except for mixed deciduous with alder where P=0.02; Table S3), while species richness in birch forests was significantly greater than in broad-leaved deciduous forests (P<0.001), lime (P=0.002) and spruce forests (P<0.001; Supporting Table S3). Shannon diversity was also greater in mixed oak and birch forests, and lowest in lime forests, although the only significant differences were between birch and spruce forests (P = 0.029), birch and broad-leaved deciduous forests (P=0.002), and between mixed oak and broad-leaved deciduous forests (P=0.004; Fig. 3a; Supporting Table S3).

For substrate type, species richness was lowest in crustose lichen substrates (1.0 +/- 0.36). However, there were few samples from this substrate (and from thallose liverworts), and

although the main model indicated a significant effect of substrate, pairwise contrasts corrected for multiple tests by the Tukey method did not show any significant differences in species richness between substrates (Supporting Table S3).

Community composition and environmental variables

We investigated if tardigrades species community composition was correlated with annual precipitation, precipitation seasonality, summer temperatures, forest types and substrate types. The constrained distance-based redundancy analyses showed divergence in tardigrade community composition between substrate types, and to a lesser degree between forest types (Fig. 5). Permutational analysis of variance of the ordination model supported this, with community composition varying with both forest type and substrate type (P<0.001), but not with climate variables (P>0.05; Supporting Table S4). The variance between forest types and substrate types did not significantly differ from homogeneity (analysis of multivariate homogeneity of group dispersion; forest: $F_{5,193}$ = 1.75, P = 0.12, substrate: $F_{6,192}$ = 1.30, P = 0.36).

DISCUSSION

Large-scale faunistic and ecological studies are uncommon for microinvertebrates, mainly due to the time-consuming activities of extraction, slide mounting and identification of specimens, but also due to the frequent scarcity of taxonomic specialists. Nevertheless, extensive faunistic and ecological studies are fundamental to increase the knowledge of biodiversity, distribution and adaptation of invertebrate species, and to assess ecological conditions in study areas. Such studies rarely involve tardigrades although exceptions exist (e.g. Bartels & Nelson, 2006; Bartels & Nelson, 2007; Bertolani & Rebecchi, 1996; Dastych, 1980; Guil *et al.*, 2009; Johansson *et al.*, 2013; Nelson *et al.*, 2020). In our project, we performed an extensive (in term of areas, samples and habitats) faunistic/ecological study on tardigrades across Norwegian boreal forests involving ecologists and expert taxonomists to cover knowledge of the tardigrade species as well as their environments and substrates (i.e.

mosses, liverworts, lichens, leaf litter). The large amount of data allowed us to increase the knowledge of the tardigrade fauna of Norway, but also to perform an exploratory ecological study on the biodiversity and community composition in relation to climate (summer temperature and rainfall regime and seasonality), environments (forest types) and habitats (substrate types).

Remarks on the recorded taxa

In the present study 47 tardigrade species were identified, including seven species new to Norway [i.e. *Astatumen bartosi* (Węglarska, 1959), *Echiniscus spiniger* Richters, 1904, *Grevenius pushkini* (Tumanov, 2003), *Itaquascon placophorum* Maucci, 1973, *Mesocrista revelata* Gąsiorek et al., 2016, *Nebularmis reticulatus* (Murray, 1905) and *Ursulinius lunulatus* (Iharos, 1966) (Table 2)]. Three recorded species, *Pseudechiniscus* (*Pse.*) *lacyformis* Roszkowska et al., 2020, *Pse*. (*Meridioniscus*) *indistinctus* Roszkowska et al., 2020 and *Paramurrayon meieri* Guidetti et al., 2022 were recently described as new to science (Guidetti *et al.*, 2022; Roszkowska *et al.*, 2020). *Astatumen bartosi*, *Echiniscus spiniger*, *Nebularmis reticulatus* and *Ursulinius lunulatus* are widely distributed species, and are also found in geographical regions outside Europe. However, this wide geographic range may suggest the existence of species complexes or incorrect identifications (Gąsiorek & Michalczyk, 2020; Kaczmarek *et al.*, 2015; Kaczmarek *et al.*, 2016; McInnes *et al.*, 2017; McInnes, 1994; Michalczyk *et al.*, 2022). *Grevenius pushkini* (Tumanov, 2003) was until present reported only from the type locality in Russia and later from Poland (Kaczmarek *et al.*, 2011a; Kosztyła *et al.*, 2016; Tumanov, 2003). Both previous records were from freshwater sediments, while the species was found in a terrestrial wet habitat in our study. This may suggest a wider habitat preference for *Gre. pushkini*, or the presence of cryptic species. *Mesocrista revelata* is a recently described species very similar to the widely distributed *Mesocrista spitzbergensis* (Richters, 1903) (Gąsiorek *et al.*, 2016), a species reported from Norway in the past (Meier, 2017). It is possible that previous reports of *Mec.*

spitzbergensis from Norway belong to *Mec. revelata. Itaquascon placophorum* is a rather rare species known only from the Holarctic region (Kaczmarek *et al.*, 2016; McInnes, 1994). A very large number of species (58) were given interim names and are considered as possibly new species to science. They belong to the genera: *Adropion, Bertolanius, Bryodelphax, Claxtonia, Diphascon, Echiniscus, Grevenius, Guidettion, Hypechiniscus, Hypsibius, Isohypsibius, Itaquascon, Macrobiotus, Mesobiotus, Milnesium, Minibiotus,, Paramacrobiotus, Pilatobius, Pseudechiniscus, Ramazzottius* and *Tenuibiotus* (for more details see Supporting Table S1). Such a large number of taxa (ca. 48% of the identified species) classified as potentially new to science suggests that not only the Norwegian tardigrade fauna, but also tardigrades in general, are still very poorly known.

Twenty-five taxa could not be identified to species with certainty (Table 2; Supporting Table S1) due to missing eggs, the presence of few specimens not showing diagnostic characters, or the lack of a clear taxonomic diagnosis at the species level.

Biodiversity of different forest types and substrates

The main results from the analyses of biodiversity indices were that large-scale ecological variables (climate and forest type) were more important in determining the diversity of tardigrades than smaller-scale variables (substrate type) across boreal forests in Norway. For both species richness and the Shannon index there were significant differences between forest types, with the mixed oak forest having the highest tardigrade diversity and lime forest the lowest, despite the lower number of analysed samples and lower diversity of substrate types from the mixed oak forest.

In contrast to forest type, substrate type (classified by growth form or as leaf litter) was a weak predictor of recorded biodiversity, with significant effect only on species richness and a lack of significant differences in the pairwise comparisons. Thus, we found no consistent differences in the diversity of tardigrades among different growth forms of mosses, liverworts, lichens, or leaf litter. Similar results were reported from a large study in the Great Smoky Mountains National Park (USA) where no differences in estimates of tardigrade diversity,

including species richness and Shannon index, were found between mosses and lichens (Nelson *et al.*, 2020). Instead, the strongest environmental predictor of tardigrade diversity in mosses of the Great Smoky Mountains was SOx deposition, with lower tardigrade diversity in sites with high SOx deposition levels. In contrast, Zawierucha *et al.* (2017), in a smaller scale study, showed that in Arctic mosses at seabird colonies, the number of tardigrades were higher than in lichens. Even if the pairwise comparisons between substrates did not reveal any significant differences after multiple-comparison corrections in our study, the overall analysis indicated that there were differences between the substrates. For species richness, pleuorocarpous mosses and leafy liverworts had the highest median values, and crustose lichen the lowest. High abundance and species richness of tardigrades in pleurocarp mosses was also reported by Jönsson (2003) in a south-Swedish spruce forest. Pleurocarp mosses are characterised by complex architecture which may facilitate high species diversity through habitat diversity. Analyses of tardigrade abundance and biodiversity in plant substrates of different growth forms are scarce, in fact our study may represent the first large-scale analysis of tardigrade diversity related to growth forms in bryophytes and lichens.

Annual precipitation was positively related to higher tardigrade biodiversity, although the effect was weak and variation in diversity high across the range of precipitation. A positive relationship between tardigrade species richness and annual precipitation has also been found in other studies, e.g., by Guil *et al.* (2009) in a study on rock mosses and leaf litter in Spain.

Patterns of community composition

Our results showed that the composition of tardigrade communities in boreal forests in South and Central Norway is determined by both forest and substrate type. It is interesting that substrate types (with variable micro-environments) are not the only factors influencing tardigrade communities, and that forest types also influence the community structure. Differences in species composition among moss samples of different forests have previously been recorded in Poland by Dastych (1988). Although the precise variables involved are

difficult to identify, it is likely that overall characteristics of the forests' macro-environments influence the presence of the given species in the different substrates. Similar patterns have been demonstrated in soil fauna where presence and abundance of tree species affect community composition (Korboulewsky *et al.*, 2016), and in amphibians where forest type was found to be more important than site in predicting both species composition and abundance (von May *et al.*, 2010).

The characteristics of the substrate (in this study identified by eight different typologies) influence the species community, indicating that the kind of moss, liverwort or lichen is important for the presence of certain species communities. A smaller scale study, at one of the study sites, found differences in community composition among moss, lichen and leaf litter substrates using DNA metabarcoding, although some species were common in all three habitats (Topstad *et al.*, 2021). Using the same approach, Arakawa (2020) recorded differences in communities in substrates as similar as two xeric and mesic mosses from Japan. This is interesting in light of a study of boreal mosses showing differences in bacterial communities among species at the forest floor (Holland-Moritz *et al.*, 2021), meaning that bacteriovorous tardigrades experience differences in habitat quality at a very fine scale. The existence of specific communities and/or taxa associated with certain substrates has been previously reported for tardigrades (e.g. Bartels & Nelson, 2013; Bertolani & Biserov, 1996; Guidetti *et al.*, 1999; Guil *et al.*, 2009; Guil & Sanchez-Moreno, 2013; Nelson *et al.*, 2020; Nelson *et al.*, 2010; Ramsay *et al.*, 2021; Young *et al.*, 2018) and for other micrometazoans such as rotifers (Fontaneto *et al.*, 2006; Fontaneto *et al.*, 2011). As far as we know, there are only a few studies that found no differences in tardigrade communities among moss species (e.g. Kathman & Cross, 2011; Nelson, 1975). As documented in our study and reported by Nelson *et al.* (2018) and Ramsay *et al.* (2021), it is probable that growth form is more important for the community composition than the living substrate (e.g., a lichen or a bryophyte).

In our study, the leaf litter community differed more from bryophyte and lichen communities than the bryophyte and lichen communities differed among each other (Fig. 5). This

compares well with previous findings (Nelson *et al.*, 2020; Topstad *et al.*, 2021). The peculiarities of tardigrades communities within leaf litter were explored by Guidetti *et al.* (1999), who showed that leaf litter of beech forests in temperate areas of different continents have similar community compositions, and by Guil & Sanchez-Moreno (2013) who identified different community compositions in different types of leaf litter in a more restricted area. The divergent tardigrade communities found in different substrates underline that the species have ecological adaptations for specific habitats, although moss and lichen communities seem more similar to each other than to leaf litter communities. Adaptations to specific habitats are supported by morphological traits in some species; for instance in soil tardigrades that have short legs and claws and elongated bodies (Bertolani & Biserov, 1996), and in freshwater species that generally have longer claws with respect to their terrestrial relatives (Nelson *et al.*, 2015; Kaczmarek *et al.*, 2020c;).

CONCLUSIONS

Tardigrade diversity and community composition are affected by environmental factors at different scales. Species richness appears to be more affected by large-scale ecological variables (climate and forest type) while community composition appears more influenced by small-scale ecological variables such as substrate type.

Although several taxa could not be assigned Linnean names at the species level, our exploratory study reveals interesting results on the ecology of the tardigrades communities and their diversity. Our conservative approach in the community composition analyses, removing taxa of uncertain identity at the species-level, might have masked a stronger pattern of community differences among forests and substrates. Nevertheless, some significant differences associated with biotic and abiotic factors are discovered that support previous findings for tardigrade preferences. Using "growth form" to define the substrate (as opposed to taxonomic groups) is a key step to improve our knowledge of the relationships between species and substrates as this is related to the more complex three-dimensional structure, chemical composition and water retention of the substrates. A stronger focus on

fine-scale differences in environmental conditions is needed to really understand the diversity and ecology of tardigrades and other microfaunal organisms.

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SUPPORTING INFORMATION

Supporting Figure S1. dbRDA Bray-Curtis dissimilarity in identified samples.

Supporting Table S1. Data about samples and identified tardigrades species.

Supporting Table S2. Model output for final selected models of tardigrade species richness and Shannon diversity.

Supporting Table S3. Pairwise contrasts between all factor levels retained in final models.

Supporting Table S4. Permutational analysis of variance of distance based redundancy analysis of tardigrade community composition.

Data Availability Statement

The data underlying this article are available in the article and in its online supplementary material.

Table 1. Number of analysed samples from different localities, forest types, and general substrate type.

Locality name	Forest type	Latitude/ Longitude	Elevation (m a.s.l.)	Bryophyte samples	Lichen samples	Leaf litter samples
Oppkuven	Old spruce	60.08702/10.52638	680	10	10	10
Heimseterfjell	Old spruce	60.04565/9.42790	494	10	10	10
Dælivannet	Calcareous lime	59.91754/10.53949	132	8	8	6
Elnestangen	Calcareous lime	59.80103/10.50001	33	8	7	6
Bøsnipa	Calcareous lime	59.77001/10.4711	72	8	7	6
Blekebakken	Calcareous lime	59.0551/9.67679	47	7	7	6
Åsstranda	Mixed deciduous/ calcareous lime	59.09406/9.64945	6-34	7	7	6
Skråstadheia	Nemoral mixed oak	58.19899/7.99329	28-154	7	7	7
Førland/Sletthei	Birch	58.55935/6.43991	273	8	9	6
Geiteknottane	Broad-leaved deciduous	60.11139/5.86491	126	9	8	6
Åmotsdalen	Birch	62.46105/9.43131	815	10	10	10
Gartlandselva old	Old spruce	64.54241/12.38518	88	10	10	10
Gartlandselva young	Young spruce	64.54177/12.37997	98	5	5	5
Gartlandselva cleared	Cleared spruce	64.54358/12.37926	99	5	5	5

Table 2. Taxa recorded from 321 analysed samples. Alphanumeric numbers in interim names refer to sample numbers and initials of the person first recording the morphotype (* - species probably new to science; ** - species described as new to science during present study; *** - species new to Norway).

Class	Family	Genus	Species	Juveniles & adults	Eggs
Heterotardigrada					
	Echiniscidae	*Bryodelphax*	*Bryodelphax* cf. *parvulus*	6	0
			Bryodelphax sp. 305 LK*	4	0
		Claxtonia	*Claxtonia wendti*	172	0
			Claxtonia sp. 259 RG*	7	0
		Diploechiniscus	*Diploechiniscus oihonnae*	982	0
		Echiniscus	*Echiniscus blumi*	17	0
			Echiniscus cf. *crassispinosus*	4	0
			Echiniscus granulatus	2	0
			Echiniscus merokensis	425	0
			Echiniscus quadrispinosus	864	0
			*Echiniscus spiniger***	19	0
			Echiniscus testudo	4	0
			Echiniscus sp.	9	0
			Echiniscus sp. 135 LK*	1	0
			Echiniscus sp. 219 LT*	36	0
			Echiniscus sp. 224 LT*	27	0
			Echiniscus sp. 234 LT*	2	0
			Echiniscus sp. 262 RG*	1	0
		Hypechiniscus	*Hypechiniscus gladiator*	3	0
			Hypechiniscus sp. 263 RG*	6	0
		Nebularmis	*Nebularmis reticulatus***	162	0
		Pseudechiniscus	*Pseudechiniscus* cf. *ehrenbergi*	30	0
			*Pseudechiniscus indistinctus***	170	0
			*Pseudechiniscus lacyformis***	100	0
			Pseudechiniscus sp.	2	0
			Pseudechiniscus sp. 131 RG*	21	0
			Pseudechiniscus sp. 191 RG*	1	0
			Pseudechiniscus sp. 250 LK*	37	0
			Pseudechiniscus sp. 260 LK*	182	0

		Pseudechiniscus sp. 44 TM*	8	0
		Pseudechiniscus sp. 65 RG*	526	0
Eutardigrada - Apochela				
	Milnesiidae	Milnesium	*Milnesium* cf. *eurystomum*	27	0
			Milnesium sp. 15 TM*	20	0
			Milnesium sp. 164 RG*	1	0
			Milnesium sp. 19 TM*	218	4
			Milnesium sp. 45 TM*	301	0
Eutardigrada - Parachela				
	Adorybiotidae	*Crenubiotus*	*Crenubiotus* cf. *ruhesteini*	72	0
	Calohypsibiidae	*Calohypsibius*	*Calohypsibius ornatus*	71	1
			Calohypsibius schusteri	11	0
	Doryphoribiidae	*Grevenius*	*Grevenius pushkini***	1	0
			Grevenius sp. 263 RG*	3	0
	Eohypsibiidae	*Bertolanius*	*Bertolanius* sp. 340 LK*	13	1
	Hypsibiidae	*Adropion*	*Adropion arduifrons*	5	0
			Adropion belgicae	23	0
			Adropion prorsirostre	445	0
			Adropion scoticum	406	2
			Adropion sp. 4 TM*	20	0
		Astatumen	*Astatumen bartosi***	4	0
			Astatumen trinacriae	4	0
		Diphascon	*Diphascon chilenense*	23	0
			Diphascon pingue	869	0
			Diphascon sp. 345 LK*	5	0
			Diphascon sp. 376 RG*	30	0
		Guidettion	*Guidettion* sp. 336 TM*	1	0
		Hypsibius	*Hypsibius* cf. *microps*	48	0
			Hypsibius cf. *pallidus*	172	0
			Hypsibius cf. *seychellensis*	262	8
			Hypsibius convergens	113	0
			Hypsibius dujardini	59	0

		Hypsibius pallidus	1	0
		Hypsibius scabropygus	191	8
		Hypsibius sp.	4	0
		Hypsibius sp. 230 LT*	1	0
		Hypsibius sp. 314 TM*	1	0
	Itaquascon	*Itaquascon placophorum***	17	0
		Itaquascon sp. 234 LT*	18	0
		Itaquascon sp. 83 RG*	7	0
	Mesocrista	*Mesocrista revelata***	110	0
	Notahypsibius	*Notahypsibius pallidoides*	424	4
	Pilatobius	*Pilatobius bullatus*	10	0
		Pilatobius oculatus	156	0
		Pilatobius recamieri	1	0
		Pilatobius sp. 18 TM*	46	0
		Pilatobius sp. 325 TM*	7	0
	Platicrista	*Platicrista angustata*	82	0
Isohypsibiidae	*Dianea*	*Dianea sattleri*	272	0
	Fractonotus	*Fractonotus caelatus*	23	0
		Fractonotus verrucosus	6	0
	Isohypsibius	*Isohypsibius* cf. *reticulatus*	1	0
		Isohypsibius prosostomus	129	0
		Isohypsibius sp.	3	0
		Isohypsibius sp. 142 RG*	11	0
		Isohypsibius sp. 162 RG*	19	0
		Isohypsibius sp. 165 RG*	6	0
		Isohypsibius sp. 250 LK*	2	0
		Isohypsibius sp. 322 RG*	1	0
		Isohypsibius sp. 83 RG*	24	0
	Ursulinius	*Ursulinius* cf. *austriacus*	14	0
		*Ursulinius lunulatus***	2	0
		Ursulinius sp.	1	0
Macrobiotidae	*Macrobiotus*	*Macrobiotus* cf. *echinogenitus*	15	5

	Macrobiotus cf. *hannae*	0	1
	Macrobiotus gr. *hufelandi*	1212	4
	Macrobiotus hufelandi	3130	519
	Macrobiotus macrocalix	447	81
	Macrobiotus vladimiri	126	11
	Macrobiotus sp.	7	0
	Macrobiotus sp. 106 RG*	0	1
	Macrobiotus sp. 201 RG*	1	0
	Macrobiotus sp. 236 RG*	9	1
	Macrobiotus sp. 305 LK*	0	1
	Macrobiotus sp. 321 RG*	0	1
	Macrobiotus sp. 51 TM*	14	5
	Macrobiotus sp. 60 TM*	99	18
	Macrobiotus sp. 92 RG*	169	63
Mesobiotus	*Mesobiotus* cf. *coronatus*	131	48
	Mesobiotus montanus	147	46
	Mesobiotus sp.	354	0
	Mesobiotus sp. 144 LK*	185	70
	Mesobiotus sp. 236 RG*	49	29
	Mesobiotus sp. 259 RG*	11	1
	Mesobiotus sp. 272 LK*	34	20
	Mesobiotus sp. 351 LK*	25	3
	Mesobiotus sp. 57 TM*	13	7
Minibiotus	*Minibiotus intermedius*	364	3
	Minibiotus sp. 164 RG*	1	0
	Minibiotus sp. 242 LK*	146	2
Paramacrobiotus	*Paramacrobiotus* gr. *areolatus*	1	0
	Paramacrobiotus gr. *richtersi*	44	0
	Paramacrobiotus sp. 345 LK*	0	2
	Paramacrobiotus sp. 55 TM*	9	7
	Paramacrobiotus sp. 86 LK*	123	81
Sisubiotus	*Sisubiotus spectabilis*	2	2

	Tenuibiotus	*Tenuibiotus* sp. 169 LK*	1	0
Microhypsibiidae	*Microhypsibius*	*Microhypsibius truncatus*	10	0
Murrayidae	*Dactylobiotus*	*Dactylobiotus* sp.	1	0
	Paramurrayon	*Paramurrayon meieri***	770	47
Ramazzottiidae	*Hebesuncus*	*Hebesuncus conjungens*	42	2
	Ramazzottius	*Ramazzottius* cf. *oberhaeuseri*	3	2
		Ramazzottius sp. 108 LK*	13	0
		Ramazzottius sp. 111 RG*	8	0
Total			**16363**	**1111**

Table 3. Number of species recorded in substrate types by the major forest types.

Substrate\Forest	Broad-leaved deciduous	Mixed deciduous w/alder	Birch	Mixed oak	Spruce	Lime	Grand total
Leaf litter	10	9	46	20	29	30	**72**
Crustose lichen		2				4	**5**
Foliose lichen	24		29	22	31	39	**75**
Fruticose lichen	7		35	1	21	10	**48**
Unclassified lichen				8			**8**
Leafy liverwort	7	6	5		23	5	**33**
Thallose liverwort						2	**2**
Acrocarpous moss		7	17	17	9	8	**33**
Pleurocarpous moss	25	10	38	27	37	29	**70**
Grand total	**42**	**22**	**78**	**41**	**55**	**65**	**131**

Figure legends

Figure 1. Geographical positions of sampled forests in Norway. The background colours across the three panels represent the three main bioclimate gradients. Precipitation seasonality is represented as the standard deviation of monthly average precipitation.

Figure 2. Species richness (top) and Shannon diversity (bottom) plotted against gradients of summer temperature (mean temperature of the warmest quarter, left), precipitation (annual

average, centre) and precipitation seasonality (standard deviation of monthly average precipitation, right). Points are jittered to facilitate visualisation of overlapping values.

Figure 3. Boxplots showing distribution of species richness (top) and Shannon diversity index (bottom) between forest types. Boxes show the interquartile range (IQR), the lines show the median value and whiskers 1.5x IQR, and dots represent values outside this range. Numbers in the boxes show the number of samples per forest type.

Figure 4. Boxplots showing distribution of species richness (top) and Shannon diversity index (bottom) for different substrate types. Boxes show the interquartile range (IQR), the lines show the median value and whiskers 1.5x IQR. Dots represent outliers. Numbers in the boxes show the number of samples per substrate.

Figure 5. Constrained ordination of tardigrade communities (distance-based RDA). The 95% confidence ellipses of the centroid location are shown. In the left panel points and ellipses are coloured by substrate, while in the right panel, points and ellipses are coloured by forest type.