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Potential influence of birds on soil testate amoebae in the Arctic

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ABSTRACT

Birds can be an important agent of environmental change in High Arctic ecosystems, particularly due to the role of seabirds as a vector transferring nutrients from the marine to terrestrial realms. The soils of bird nesting sites are known to host distinct plant communities but the consequences of bird modification for microorganisms are much less clear. Our focus here is testate amoebae: a widely-distributed group of protists with significant roles in many aspects of ecosystem functioning. We compared the testate amoeba assemblages of a site on Spitsbergen (Svalbard archipelago) affected by nesting birds, with nearby control sites. We found differences in assemblage between sites, typified by reduced relative abundance of *Phryganella acropodia* and *Centropyxis aerophila* in bird-modified soils. These changes may reflect a reduced availability of fungal food sources. We found no evidence for differences in assemblage diversity or test concentration between bird-modified and control soils. Our dataset is small but results provide the first evidence for the potential effect of bird modification of soils on testate amoebae in the Arctic. Results show only limited similarity to experimental studies of nutrient addition, implying that response mechanisms may be more complicated than simply additional nutrient supply through faeces.

Keywords: Birds; Testate amoebae; Protists; Protozoa; Svalbard

1. Introduction

High Arctic terrestrial ecosystems typically have very low rates primary production but adjacent seas can have relatively high productivity, at least during ice-free periods. Seabirds are an important vector transporting nutrients from the marine realm to terrestrial ecosystems, depositing them in the form of guano, eggshells, feathers, and carcasses (Zwolicki et al., 2013). This link may assume additional significance under climate change with retreating sea ice facilitating large phytoplankton blooms in Arctic seas (Arrigo et al., 2008; Yool et al., 2015). Other birds may have important roles in redistributing nutrients within and amongst Arctic terrestrial habitats. Bird colonies can form important nutrient 'hotspots' in nutrient-poor Arctic environments with bird-modified soils often enriched in key limiting nutrients including N, P, Mg and K (Ligeza and Smal, 2003; Wait et al., 2005; Zwolicki et al., 2013). This supply of nutrients may ultimately lead to the development of soils which are higher in moisture, more acidic and more saline than those of areas not affected by birds (García et al., 2002; Wait et al., 2005). Biological consequences of these changes include the tendency for bird-modified soils to host plants with more nutrient-rich tissues and plant communities with lower species richness, higher biomass, greater cover and domination by a smaller pool of ruderal, annual and cosmopolitan species (Anderson and Polis, 1999; Ellis, 2005). Bird-modified soils often host distinct micro-fauna assemblages with effects potentially extending some distance beyond nesting sites (Zawierucha et al., 2016; Zmudczyńska-Skarbek et al., 2017; Zmudczyńska-Skarbek et al., 2015). The combination of these varying impacts may ultimately have an important role in shaping long-term ecosystem development in High Arctic environments (Hodkinson et al., 2003; Sánchez-Piñero and Polis, 2000). With many direct and indirect anthropogenic factors currently acting on Arctic bird populations there is a need to understand how birds shape terrestrial ecosystems and how this may vary in the future (Moe et al., 2009; Stempniewicz et al., 2007; Wassmann et al., 2011).

While the consequences of bird-modification have been comparatively well-studied for plants, the consequences for the microorganisms which constitute the largest component of biodiversity are virtually unknown. Previous studies imply that birds increase total microbial biomass and, consequently soil respiration, and shift the competitive balance from fungi to bacteria, but other impacts are unclear (Smith, 2003, 2005; Wright et al., 2010). Birds may affect soil microbial groups through several pathways, both directly through the supply of nutrients and physical disturbance, and indirectly through impacts on prey, predators and competitors. Birds are also important agents in the movement of small organisms and given long ranging distances may be a vector for the transport of microorganisms to nesting sites (Wilkinson, 2009; Wilkinson et al., 2012). While there is basic knowledge about the effects of bird populations on overall microbial biomass and some

knowledge about changes in prokaryotes there is almost no knowledge about the response of microbial consumers despite their important role in shaping microbial food-web dynamics and ultimately many aspects of ecosystem function.

The particular focus of this paper is testate amoebae. Testate amoebae are an abundant group of protists in soils and can constitute a large proportion of total microbial biomass (Gilbert et al., 1998b). These microorganisms are amongst the larger protist groups (most taxa 20-200µm in length) and play import roles as consumers of smaller microorganisms and, in the case of some species, in primary production through endosymbiotic algae (Jassey et al., 2015; Wilkinson and Mitchell, 2010). While it is probable that general ecological studies of protozoa in high latitude environments have included samples from bird-modified soils (Smith, 1996; Vincke et al., 2004), to our knowledge the only study to directly address the impact of birds on soil testate amoebae is that of Vincke et al. (2007) from sub-Antarctic Île de la Possession (Crozet Archipelago). This study showed significant changes to testate amoeba assemblages but results are difficult to generalise to other sites because the study focused on the impact of wandering albatross which is restricted to the southern hemisphere and is an atypical bird species in terms of size and time spent on land. We are not aware of studies investigating the impacts of other bird species or any studies at all in the Arctic. However, numerous studies demonstrate impacts of various aspects of nutrient enrichment on soil testate amoebae (Mitchell, 2004; Payne et al., 2013; Payne et al., 2012) so it is reasonable to suppose that birds are affecting soil testate amoebae widely across the Polar regions. Here we address differences in testate amoebae between sites with differing extents of bird modification on the island of Spitsbergen in the Svalbard archipelago. We hypothesise that:

H1 Areas affected by birds sites host distinct assemblages of testate amoebae from adjacent sites.

H2. Areas affected by birds host exotic taxa not present in the wider environment due to avian transport.

H3 Areas affected by birds host a higher abundance of bacterivorous and lower abundance of fungivorous taxa relative to reference sites.

H4. Areas affected by birds have a lower alpha diversity of testate amoebae.

2. Material and methods

2.1. Study area

The Svalbard archipelago is located in the Arctic between 74° and 81° N and 10° and 35° E, at a distance of c.700 km from the nearest continental coast. The archipelago covers an area of about 63,000 km^2, 60% of which is covered with ice and snow (Hisdal, 1985). Our research was conducted in the Grønfjorden area of Western Spitsbergen, in the vicinity of the settlement of Barentsburg (78° 02'N, 14° 12' E) in July 2012 (Fig. 1). The climate of Svalbard is atypically mild for the latitude because of northwards transfer of heat by the North Atlantic Drift. The nearest meteorological station with available data is located >50 km from our sampling area at Longyearbyen Airport. Here the average annual temperature is -4.6°C (1981-2010 average) with positive average temperatures recorded only in the summer months (June to September) (Førland et al., 2011). The monthly average air temperature in winter is often below -15°C, but the daily minimum temperatures can drop to -40°C. Precipitation, mostly as snow, varies from 210 to 525 mm per year (1981 – 2010). The study area is underlain by permafrost; monitoring data show active layer depths of 1-2m (Osokin and Sosnovsky, 2008).

2.2. Fieldwork and sampling sites

Sampling was conducted in three different sites: one affected by birds ('bird site') and two nearby control sites without major bird influence ('control sites 1 & 2'). The bird site is a slope situated immediately below a nesting site for *Alle alle* (Little Auk), also with *Cepphus grille* (Black guillemots), *Larus hyperboreus* (Glaucous Gull), and occasionally *Branta leucopsis* (Barnacle Goose). The colony is smaller than a typical Little Auk colony on Svalbard, containing no more than 500 pairs at the time of the sampling visit. In 2005 and 2008, bird numbers were quantified as around 200-250 pairs of Little Auks, around 20-25 pairs of Black Guillemots, 2-6 pairs of Barnacle Geese and 14-17 pairs of Glaucous Gulls (Ivanenko, 2006; Ivanenko, 2009). Although nest density is not particularly high, faeces and guano deposits were widely noted in the sampling site which occupies a slope between 53 and 134m above sea level, situated immediately below the main colony on the seaward side (Supplementary Fig. 1). Vegetation is discontinuous and includes a range of vascular plants and bryophytes with *Polytrichum* spp. and *Sanionia uncinata* particularly abundant (Fig. 1; Supplementary Fig. 1). Towards the base of the slope the vegetation includes shrubs such as *Salix polaris*, in the centre of the slope are forbs such as *Cerastium alpinum* and *Potentilla hyparctica* while the upper part of the slope vegetation is primarily of mosses. Control site 1 is located along the same coastline ca. 2km to the south on a slope between 66 and 88m asl. This site is less steeply sloping than the bird site with deeper humus-rich soils and more continuous mixed bryophyte-dominated vegetation typical of Arctic tundra. The vegetation was not surveyed in detail but contains no clear indication of nutrient enrichment and is distinct from the bird site. No nests or

guano were noted at this site although a small amount of geese faeces was noted. Control site 2 is located further south on flatter, lower elevation (6-44m) tundra at Cape Finneset. Vegetation includes more lichens than the other sites but in other respects the site is similar to control site 1 with broadly-typical bryophyte-dominated tundra vegetation. A few nests of Snow Bunting (*Plectrophenax nivalis*) and Common Eider (*Somateria mollissima*) were noted but these were sparse and faecal deposits were much rarer than in the bird site. All three sites are beyond the boundaries of the settlement of Barentsburg and have not been significantly affected by anthropogenic disturbance through industrial or agricultural activity and are located well away from sites with imported soils (Coulson et al., 2013). It is however possible that bird distribution may have been different prior to the construction of Barentsburg (1920s) and that soils may exhibit legacy-affects from previous bird-derived nutrient addition. Five samples of topsoil and plants (5g each) were extracted from each sampling area spanning the range of variability in vegetation and microtopography. Sampling was conducted using a transect design with these transects perpendicular to the coastline in the bird site and control site 1 and parallel to the coastline in control site 2. Sampling locations were separated by distances of 10-200m and were 50-500m from the coast. Samples extracted were stored refrigerated until analysis (Mazei et al., 2015).

2.3. Laboratory work

Samples were prepared for microscopy following the method of Mazei and Chernyshov (2011) which comprises suspension in water, physical agitation and settling. All samples were inspected under light microscopy at 160× magnification. All testate amoeba tests in the samples were identified following Mazei and Tsyganov (2006). Live individuals were not differentiated from empty tests so the assemblage identified integrates communities living over a period of several years. Data are expressed as relative abundance; as a percentage of the total count of all tests. A second sample from the same location was weighed, oven dried and reweighed to calculate moisture content, which is known to be an important control on testate amoebae.

2.4. Data analysis

To address the adequacy of sampling in capturing the full amoeba community, at a sample level and overall, we used individual rarefaction to consider change in taxon richness with count and sample rarefaction of the entire dataset to assess how taxon richness changed with number of samples considered (Colwell et al., 2004). To assess the impact of bird presence we considered both the assemblage composition and measures of assemblage diversity. We conducted separate analyses of data based on both test concentration (ind. g^{-1}) and relative abundance (%). To test for

differences in assemblage between bird and control sites we used one-way permutational analysis of variance (PERMANOVA) with 9999 permutations, based on Bray-Curtis dissimilarity matrices of raw data (Bray and Curtis, 1957). To visualise differences we used a detrended correspondence analysis (DCA) ordination (Hill and Gauch, 1980). We tested for differences in morphospecies richness, test concentration and moisture content between the sampling sites using Kruskal-Wallis tests with Bonferroni-corrected pairwise Mann-Whitney post-hoc tests. We also calculated Shannon diversity H' based on the natural logarithm (DeJong, 1975). Data analysis was conducted in PAST vers. 3.04 (Hammer et al., 2001).

3. Results

3.1. Composition and taxon richness

Twenty six taxa were found in the fifteen samples (Table 1) with taxon richness by sample varying from 9 to 17 (mean=13). Shannon H' varied from 1.8-2.4. The most abundant identified taxa were *Trinema lineare* (19% of all tests counted), *Centropyxis aerophila* (15%), *Phryganella acropodia* (12%) and *Centropyxis sylvatica* (11%). Count totals for some samples were less than commonly-applied minima (mean=65 tests) (Payne and Mitchell, 2009) but individual rarefaction plots show that although an asymptote is not reached there is a marked reduction in rate of increase for most but not all samples (Fig. 2). At the level of the entire study area, the rarefaction curve similarly shows no asymptote but a strong reduction in gradient towards the maximum (Fig. 3). The sampling is likely to have captured most but not all testate amoeba taxa in this area.

3.2. Differences with bird presence

PERMANOVA showed highly significant differences between sites (relative abundance: $F_{(1.1,1.8)}$=4.1, P<0.001; concentration: $F_{(1.6,2.4)}$=2.6, P<0.001). Pairwise testing showed significant differences amongst all three sites with the most significant differences between the bird site and control site 1 (P=0.022) and marginally significant differences with control site 2 (P=0.047) and between the two control sites (P=0.049). The DCA plots illustrate these differences (Fig. 4). In terms of concentration the bird samples are clearly separated from control sites along DCA2 (Fig. 4A) while in terms of relative abundance the samples are separated along DCA1 and, to a lesser extent, DCA2 (Fig. 4B). The samples from both control sites are more tightly clustered in the ordination plots based on both relative abundance and concentration. Four taxa were only found in bird sites: *Cryptodifflugia oviformis, Cyclopyxis eurystoma, Nebela parvula*, and *Phryganella hemisphaerica* while three taxa were only found in control sites: *Euglypha simplex, Centropyxis cassis,* and

191 *Centropyxis elongata* (Table 1). The majority of these taxa were rare overall. Of the taxa with
192 sufficient data for separate testing only two showed significant differences in relative abundance
193 between sites: *Phryganella acropodia* (N=15, df=2, H=7.22, P=0.03) and *Centropyxis aerophila* (N=15,
194 df=2, H=12.5, P=0.001). *C. aerophila* contributed a large proportion of all tests in the control sites (9-
195 46%) but was rare in the bird sites (0-4%) (Fig. 5). *P. acropodia* relative abundance in the bird site
196 was significantly lower than control site 2 ($P<0.05$) but not significantly lower than control site 1 (Fig.
197 5). We found no significant difference in moisture between samples from the three sites (N=15,
198 df=2, H=1.1, P=0.56)(Fig. 6). There was no significant difference between sites in terms of testate
199 amoeba morphospecies richness (N=15, df=2, H=4.5, P=0.10) but the highest individual values were
200 found in the bird site (Fig. 7). There was no significant difference between the sites for total test
201 concentration (N=15, df=2, H=0.14, P=0.93) (Fig. 8).

202

203 4. Discussion

204 With only fifteen samples our dataset is undeniably small but constitutes the first direct
205 comparison of testate amoebae in bird-modified and control soils in the Arctic. The dataset is also
206 one of comparatively few for testate amoebae from the region. Svalbard is one of the better-studied
207 High Arctic regions for testate amoebae but protozoa are still clearly an under-recorded group
208 (Coulson et al., 2014). Our dataset extends the geographic coverage and the known species pool. Of
209 the taxa identified, most are well-known, widely found in soils and have been previously recorded in
210 the Arctic (Beyens and Chardez, 1995). The most notable taxon identified is the single test (shell) of
211 *Centropyxis elongata*. We believe this is the first record of this taxon on Svalbard and one of very
212 few from the Arctic (Beyens and Chardez, 1995; Beyens et al., 1991), with most records from much
213 more temperate environments (Lüftenegger et al., 1988; Ooms et al., 2015; Ying-zhi and Yun-fen,
214 1996).

215 Our results show that there are significant differences in assemblage composition between the
216 testate amoeba communities of sites with and without birds (Fig. 4). It is probable that these reflect
217 the influence of birds on testate amoebae but it is also conceivable that our sampling sites also
218 differed in other ways not related to birds. For instance, control site 2 was at a lower elevation and
219 had flatter topography than the bird breeding site and both sites were less steeply sloping than the
220 bird site. The comparison between the bird site and control site 1 is probably the more informative
221 comparison given greater proximity and topographical situation. It is notable that we found some
222 differences between the two control sites and these are unlikely to relate to bird use. We therefore

cannot rule out the possibility that the differences in testate amoebae we find are coincidental; this is a fundamental limitation of spatial comparisons of this nature. Birds do not select their nesting sites randomly; factors such as microclimate, proximity to food sources, accessibility to predators and human disturbance may determine choice of nesting site and some of these factors may also affect the soil biota (Anderson and Keith, 1980; Forbes and Kaiser, 1994). However, it appears probable that the birds have affected the testate amoeba assemblage in the soils at these sites. Thus, our data support hypothesis 1 and imply an impact of birds on soil testate amoebae.

One possibility which we anticipated at the outset is that bird nesting sites might host unusual testate amoebae including taxa translocated from other locations and other environments (H2). Of the bird populations present at our site, both Glaucous Gulls and Little Auks over-winter largely at sea in the North Atlantic but range widely across this region (Newton and Dale, 1996). There is a small possibility that these species might translocate amoebae from more southerly regions. Barnacle Geese from Svalbard appear to over-winter primarily in the Solway Firth area of Scotland (Butler et al., 1998; Owen and Black, 1989) and there is a somewhat higher possibility that they may transport amoebae to Svalbard. However, we found little unusual in the testate amoeba fauna of these samples. Across the entire Arctic region the most abundant testate amoeba taxa are *Trinema lineare, Assulina muscorum, Centropyxis aerophila* and *Corythion dubium* (Beyens and Bobrov, 2016). All of these taxa were located in our samples and they constituted the most abundant, 8th, 2nd and 7th most abundant taxa respectively. None of the taxa we identify can be considered as a particular surprising presence in a habitat of this nature. The most notable occurrence was the single test of *Centropyxis elongata*. While this taxon has been more commonly recorded further south this test was identified at control site 1 where no birds were apparent so a bird vector seems unlikely. Given the recording of this species from Devon Island in the Canadian Arctic Archipelago (Beyens et al., 1991) it is more probable that this is an indigenous, albeit relatively rare, component of the Arctic testate amoeba fauna. Taxonomic grouping or inconsistency is also a possibility given the general morphological similarity of this taxon to other, more frequently recorded *Centropyxis* species such as *C. platystoma* (Mitchell et al., 2014; Payne et al., 2011). Our lack of evidence for unusual or exotic taxa associated with bird nesting on Svalbard parallels work on invertebrates which also found little evidence for species introductions in nesting sites (Coulson et al., 2009a), although bird transport of invertebrates has been demonstrated (Lebedeva and Krivolutsky, 2003). In the case of our study it is possible that the predominant presence of bird species which largely over-winter at sea may have reduced any species introduction and that this could have been greater in sites with other species.

Our data imply that birds have reduced the occurrence of taxa including *C. aerophila* and *P. acropodia* (the only two taxa to show significant differences in independent testing) and may have increased the abundance of *Centropyxis sylvatica*, *Assulina muscorum* and *Trinema enchelys* (although all were non-significantly different in independent testing). At the outset we anticipated that bird presence would increase the abundance of bacterivorous taxa and decrease the abundance of fungivorous taxa, based on previous findings of shifts in competitive balance from fungi towards bacteria most likely due to enhanced nutrient supply (Wright et al., 2010). Our data only partially support this expectation. *P. acropodia* is widely known as a taxon which is associated with fungi and this taxon was less abundant in bird-affected sites (Gilbert et al., 2000; Ogden and Pitta, 1990). *C. aerophila* has similarly been observed to feed on fungi (Gilbert et al., 2000) and was less abundant in soils from the bird site . These findings generally support the expectation of a reduction in fungivores, however our data only provide weak evidence for an increase in bacterivores. Although no taxa were significantly more abundant in soils with birds, the largest difference in overall relative abundance was in *Centropyxis sylvatica* which has a mean relative abundance more than three times greater in soils from the bird site than the control sites. We are not aware of any direct data on the feeding preference of this taxon but they can be expected to be similar to *C. aerophila* making it surprising to see opposing trends. Similarly, taxa which are both abundant and unambiguously bacterivores such at *Trinema lineare* do not differ between the sites (Gilbert et al., 2000). We do find a general trend towards higher abundance of *Trinema enchelys* and *Assulina muscorum* in the bird sites and these taxa are most likely bacterivorous. Overall, hypothesis 3 cannot be refuted but is only partially supported by the data.

Across a wide variety of groups of organisms it is a common finding that nutrient enrichment leads to a loss of diversity with increasing dominance by a small group of taxa (Stevens et al., 2004), we therefore anticipated that bird nesting sites would be less diverse (H4). However the data did not support our initial expectations. Our results show no significant difference between sites in terms of taxon richness. It may be the case that relatively modest nest density means that nutrient input is insufficient to facilitate dominance by a small group of taxa.

Our data show similarities and differences with previous datasets. The most directly comparable study is that of Vincke et al. (2007) from the sub-Antarctic. These authors found strong differences in testate amoebae with albatross influence, typified by increased abundance of *Difflugiella oviformis* and *Trinema lineare*. *D. oviformis* was not found in our samples whereas *T. lineare* was abundant but did not differ between sites. Vincke et al. (2007) found reduced diversity near albatross nests, again in contrast to our finding of no significant difference. The differences

between the two studies may partly reflect the intensity of disturbance involved. Vincke et al. (2007) found heavy deposition of guano leading to very large differences in nutrients, particularly phosphorous, combined with extensive physical disturbance. In our sites soil nutrients were not analysed but based on observations of the extent of faeces it is likely that nutrient input was much less than that in the sub-Antarctic sites of Vincke et al. (2007). Similarly, plant communities were not recorded in either study but disturbance appears to be more extensive in Vincke et al. (2007)'s sites than in our own.

Vincke et al. (2007) is the only study we are aware of to directly investigate the impact of birds on soil testate amoebae but other studies have addressed the impacts of nutrient enrichment. Comparison to these studies provides the possibility to evaluate the extent to which our results are concordant with a nutrient enrichment effect. In perhaps the most relevant study, Mitchell (2004) investigated the impact of N&P addition on an Alaskan tundra environment and found distinct changes in testate amoeba assemblage: *P. acropodia* and *C. aerophila* increased in relative abundance while *D. oviformis* declined. The increase in *P. acropodia* and *C. aerophila* is the opposite of what we find here while the decline in *D. oviformis* is the opposite of that found by Vincke et al. (2007). In a French peatland Gilbert et al. (1998b) found that nutrient input with both NPKCa and PKCa caused a loss of testate amoeba biomass relative to other microbial groups and Gilbert et al. (1998a) found similar results for N addition alone. In our study other microbial communities were not analysed but we found no significant difference in overall test concentrations. Payne et al. (2012) investigated the impact of N addition in a heathland soil and found a decline in relative abundance of *Corythion dubium* with N, a finding which is not paralleled in our data where this taxon was non-significantly more abundant in the bird sites. Payne et al. (2013) found that gaseous ammonia was associated with a non-significant trend towards greater testate amoeba biomass, primarily driven by increasing abundance of the largest taxa. This size-dependent response to nutrient addition was not paralleled in our data. From these comparisons it can be concluded that the response of testate amoebae to bird presence is unlikely to be a simple consequence of nutrient addition in faeces. We did not collect wider supporting data which would clearly have been helpful to fully understand the mechanisms at work. Direct data on soil nutrient composition would have allowed eutrophication effects to be more directly tested and data on other microbial groups would have allowed linkages to be explored across trophic levels. Further quantification of bird species, abundance and activity would also have been helpful given evidence for differing impacts of different bird species (Zmudczyńska-Skarbek et al., 2017). Larger and more holistic studies will be required to fully disentangle the mechanisms of impact. Nevertheless, our data provide the first

direct evidence for the effects of birds on soil testate amoebae in the Arctic and pave the way for future research.

It is clear that bird modification of soils is a significant issue in the Arctic, particularly given threats to birds from climate change, invasive species and pollutants (Coulson et al., 2009b; Gabrielsen et al., 1995). Bird influence is an important factor shaping high latitude ecosystems but current knowledge is fragmentary, particularly below-ground. Testate amoebae are just one component of the soil food-web but exemplify the limited knowledge of bird effects more widely. Our results go some way towards filling the knowledge gap but also demonstrate how little is known.

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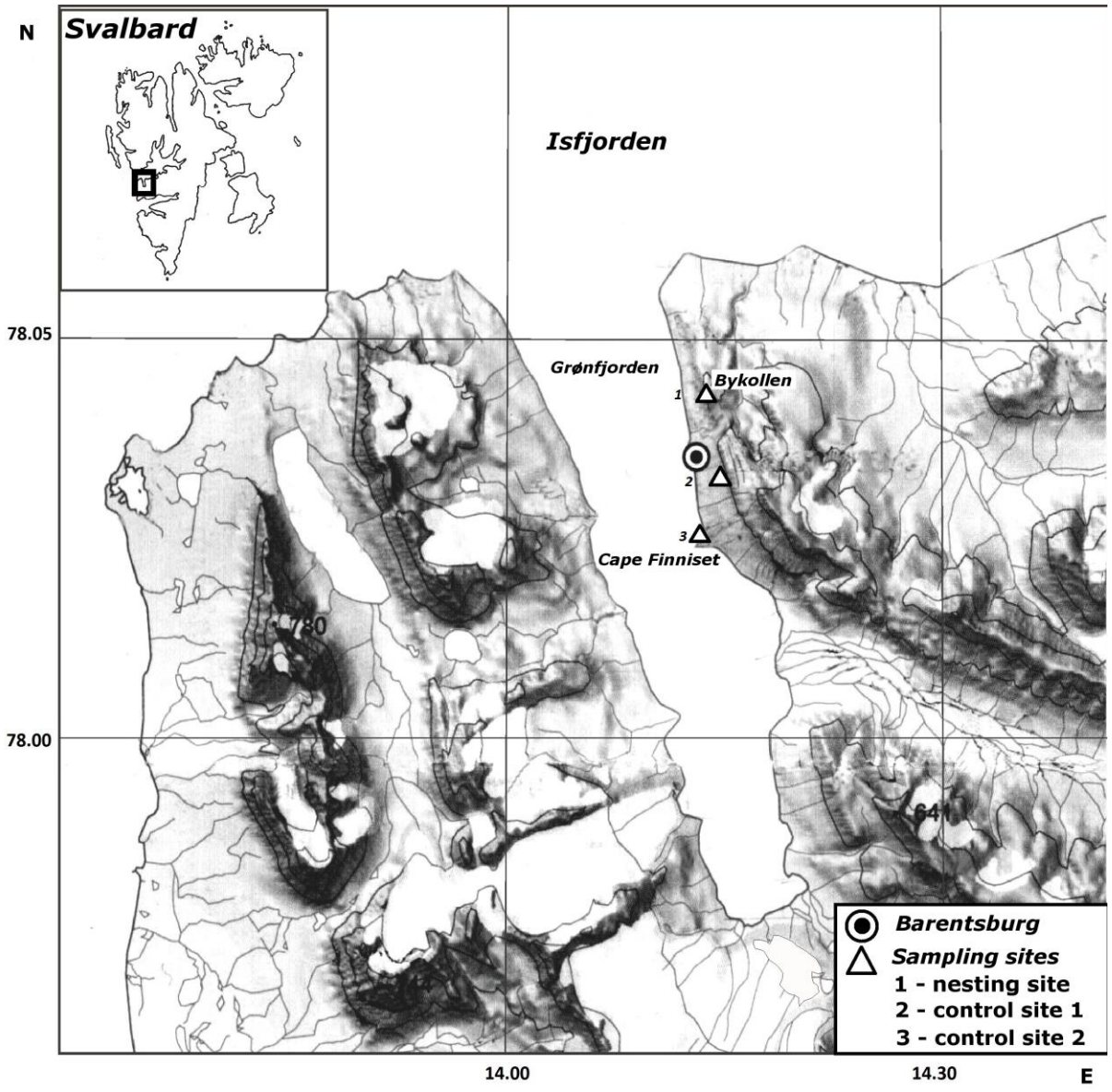
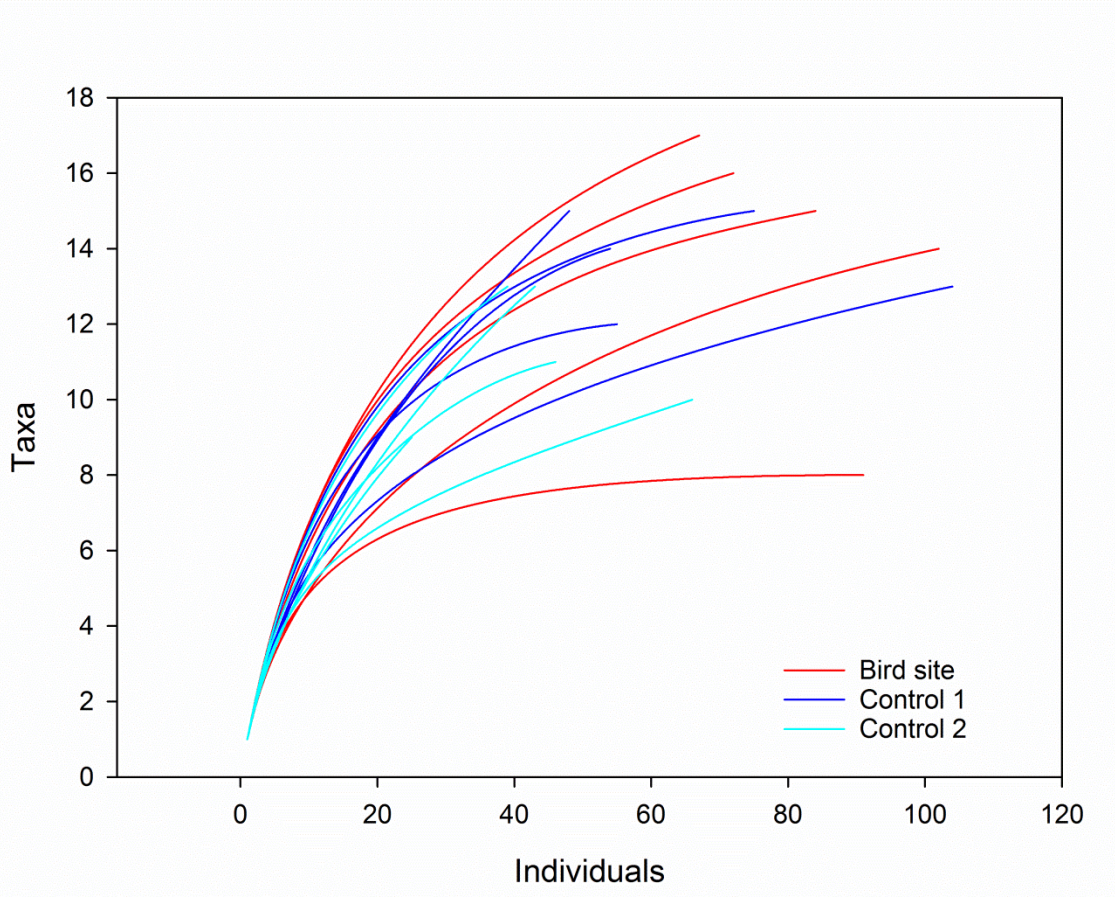
508 **Figure 1.** Location of the three sampling sites and the settlement of Barentsburg.

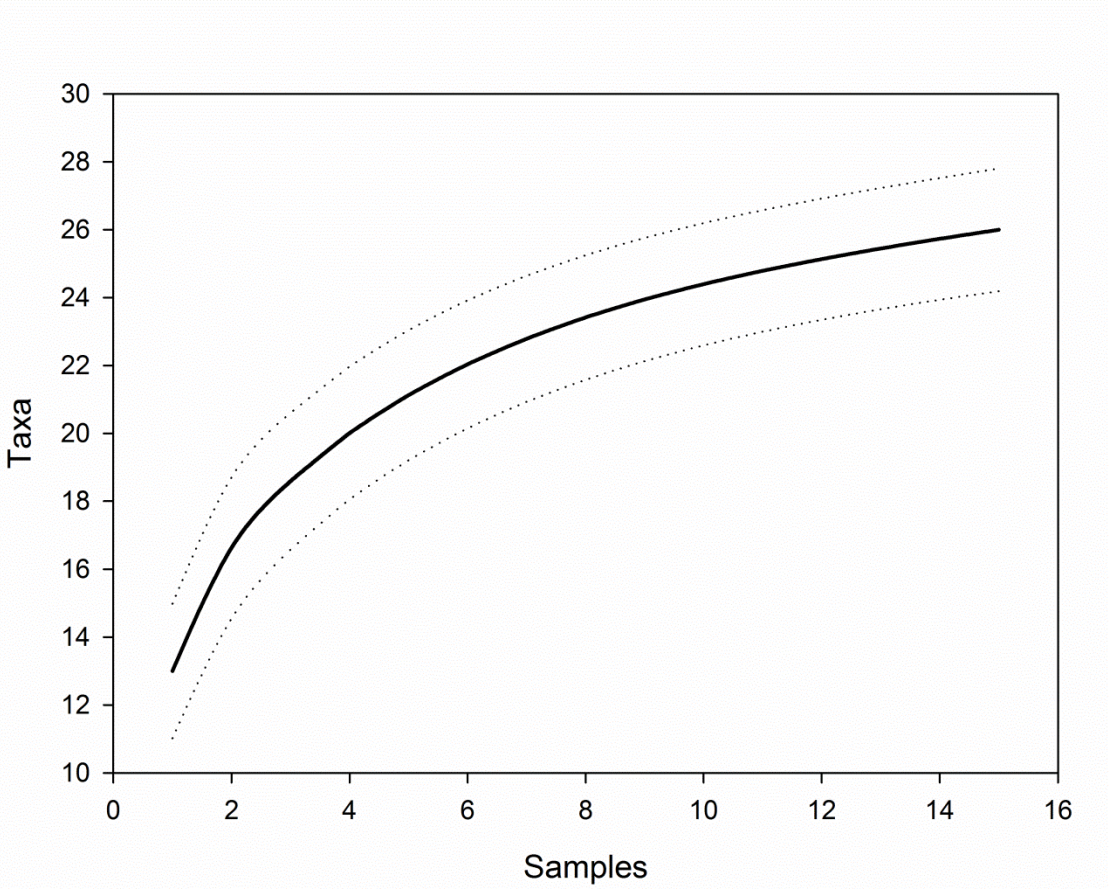


509

Figure 2. Individual sample rarefaction curves of testate amoeba composition.

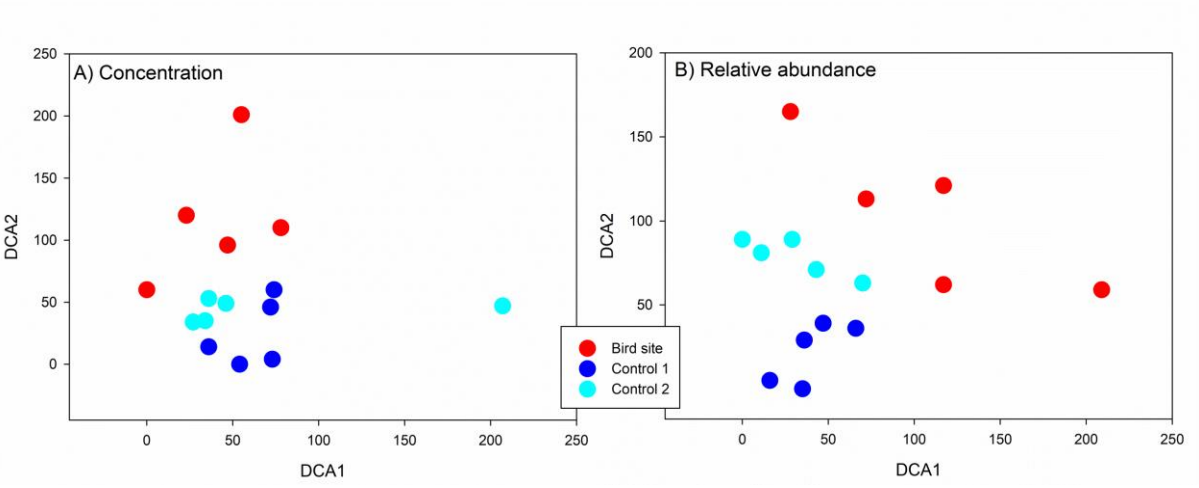


512 **Figure 3**. Overall sample rarefaction curve for entire dataset based on Mao's Tau showing standard
513 errors (dotted lines).



514

515 **Figure 4.** Detrended correspondence analysis ordination plots based on A) concentration and B)
516 relative abundance of testate amoebae.



517

518 **Figure 5.** Relative abundance of *Centropyxis aerophila* (A) and *Phryganella acropodia* (B). Box-plots
519 show median (central line), first and third quartiles (grey box) and tenth and ninetieth percentiles
520 ('whiskers'). Bars marked with differing letters show significant difference in post-hoc testing, where

two letters are shown there is no significant difference from either other.

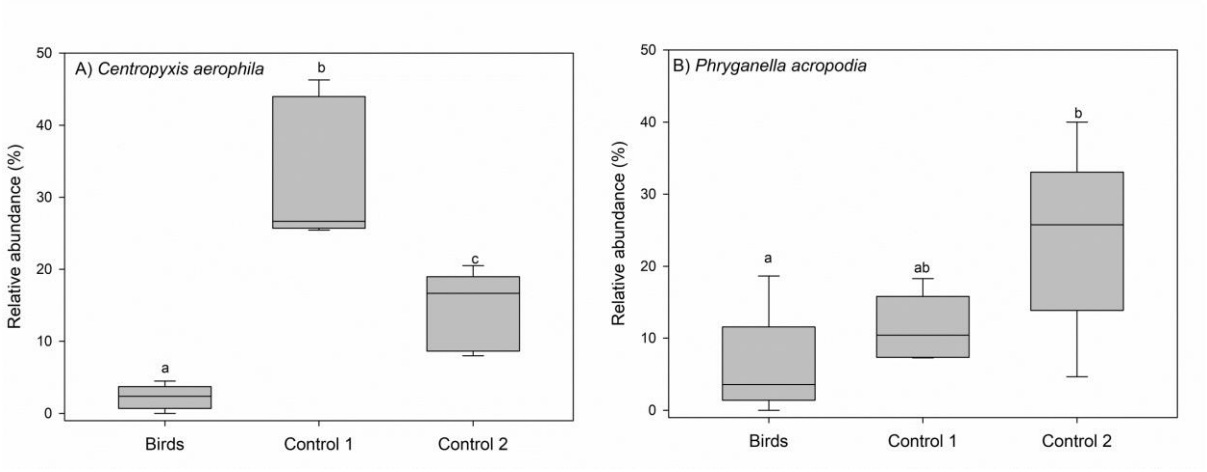


Figure 6. Sample moisture (proportion) in each of the sampling sites. Differences are non-significant (P>0.05). Box-plots show median (central line), first and third quartiles (grey box) and tenth and ninetieth percentiles ('whiskers').

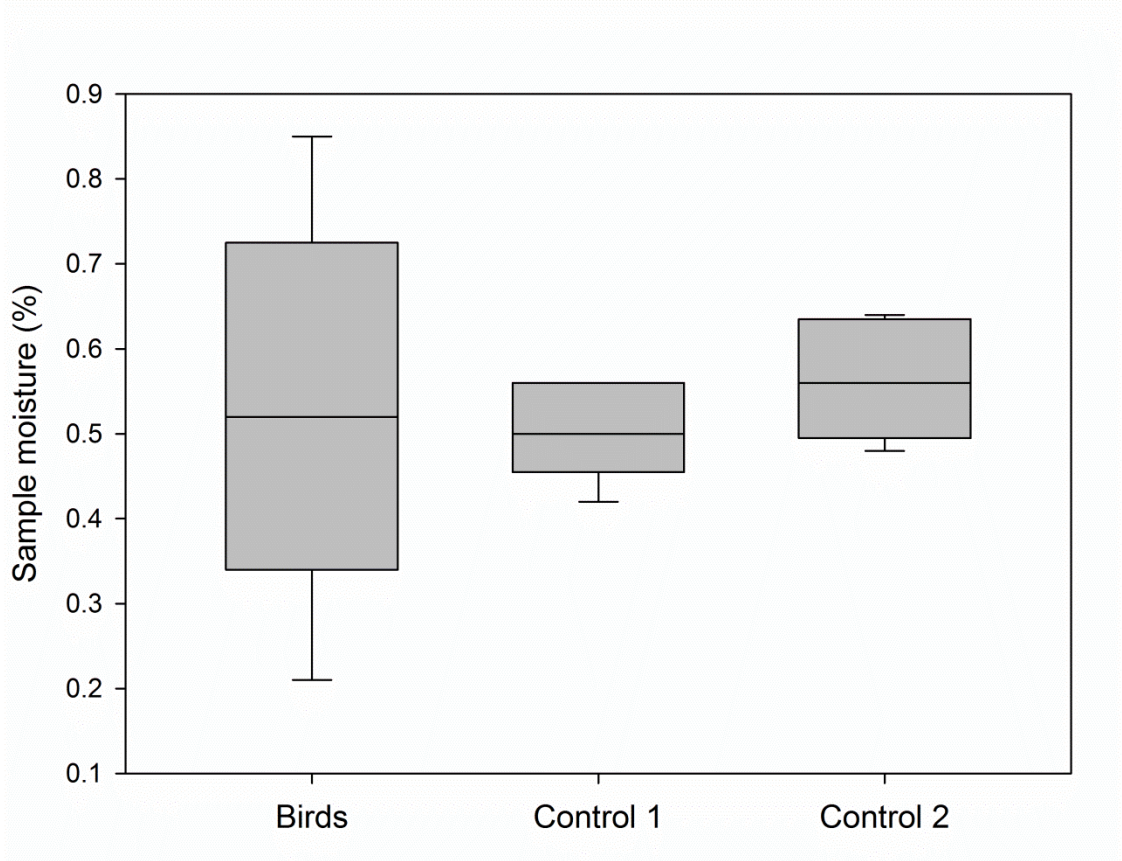
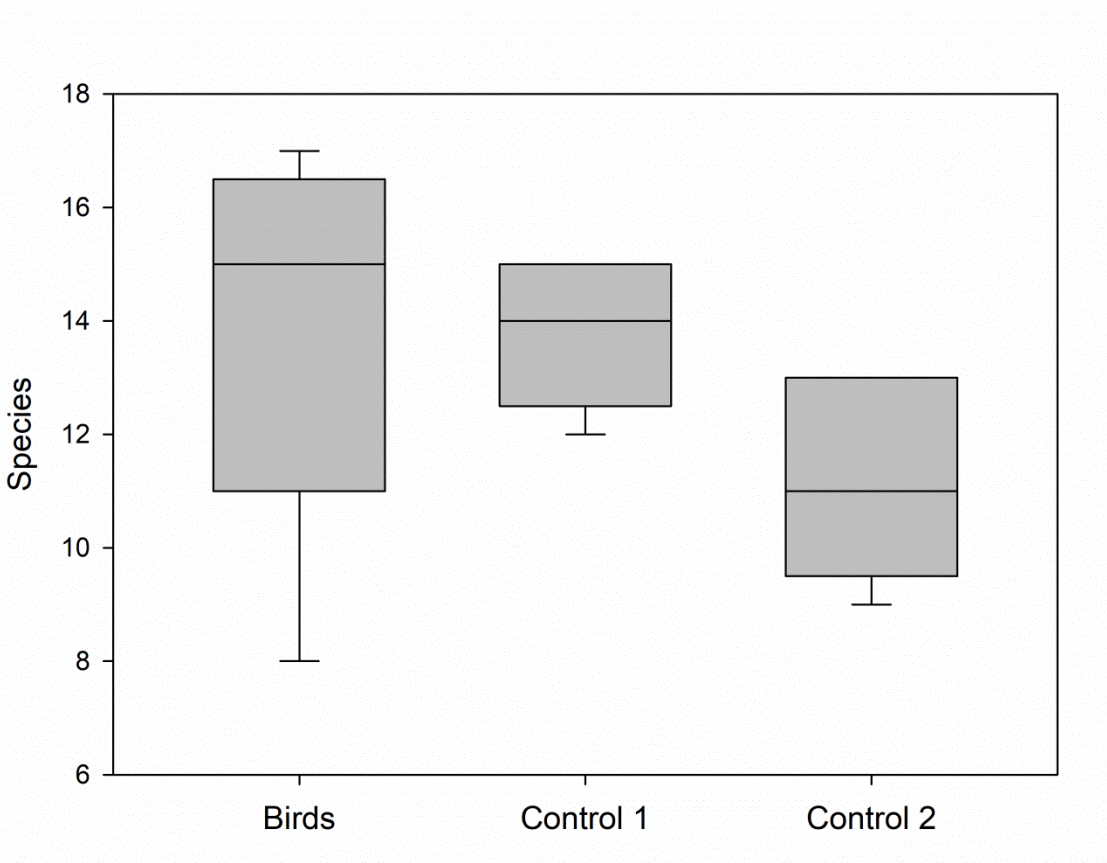


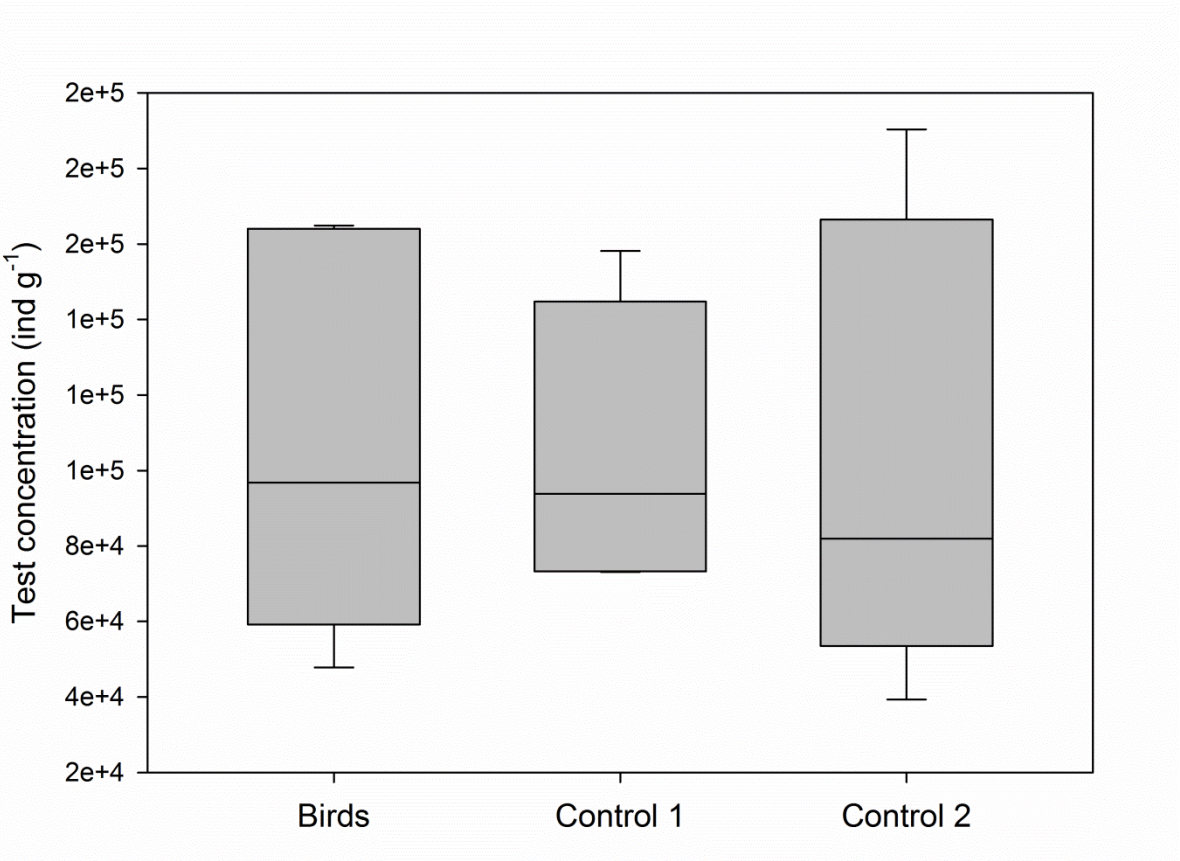
Figure 7. Morphospecies richness of testate amoebae in each of the sampling sites showing. Differences are non-significant (P>0.05). Box-plots show median (central line), first and third

529 quartiles (grey box) and tenth and ninetieth percentiles ('whiskers').



530

531 **Figure 8.** Test concentrations in each of the sampling sites. Differences are non-significant (P>0.05).
532 Box-plots show median (central line), first and third quartiles (grey box) and tenth and ninetieth
533 percentiles ('whiskers').


Test concentration (ind g-1)
2e+5
2e+5
2e+5
1e+5
1e+5
1e+5
8e+4
6e+4
4e+4
2e+4
Birds
Control 1
Control 2

534

535

536 **Table 1.** Relative abundance of all testate amoeba taxa in bird nesting site (n=5) and control sites
537 (n=10). Figures rounded to one decimal place. Figures in bold show taxa with significant (P<0.05)
538 difference between sites.

	Relative abundance (%)		
Taxon	Birds	Control 1	Control 2
Arcella arenaria var. compressa Chardez, 1974	1.2	1.2	2.3
Assulina muscorum Greeff, 1888	7.2	5.1	0.9
Centropyxis aerophila Deflandre, 1929	**2.2**	31.5	15.1
Centropyxis cassis (Wallich, 1864) Deflandre, 1929	0.0	0.0	0.5
Centropyxis elongata (Penard, 1890) Thomas, 1959	0.0	0.3	0.0
Centropyxis platystoma (Penard, 1890) Deflandre, 1929	0.2	0.6	0.9
Centropyxis sylvatica (Deflandre, 1929) Bonnet et Thomas, 1955	18.8	3.0	7.8
Corythion dubium Taránek, 1881	6.5	5.1	4.6
Corythion orbicularis (Penard, 1910) Iudina, 1996	9.6	4.8	3.2
Cryptodifflugia oviformis Penard, 1890	1.9	0.0	0.0
Cyclopyxis eurystoma Deflandre, 1929	0.7	0.0	0.0
Euglypha laevis (Ehrenberg, 1832) Perty, 1849	0.7	1.2	0.0
Euglypha rotunda Wailes, 1915	3.4	6.8	2.7
Euglypha simplex Decloitre, 1965	0.0	0.6	0.0
Euglypha strigosa var. glabra Wailes, 1898	3.1	3.0	3.7
Euglypha tuberculata Dujardin, 1841	1.7	0.3	1.8
Nebela parvula Cash, 1909	0.7	0.6	0.0
Padaungiella lageniformis (Penard, 1902) Lara et Todorov, 2012	0.2	0.0	0.0
Phryganella acropodia (Hertwig et Lesser, 1874) Hopkinson, 1909	**6.5**	12.5	22.8
Phryganella hemisphaerica Penard, 1902	1.4	0.0	0.0
Plagiopixis callida Penard, 1910	0.2	0.9	1.4
Plagiopixis declivis Thomas, 1958	1.2	0.6	0.0
Tracheleuglypha dentata Deflandre, 1928	0.2	0.0	0.9

Trinema complanatum Penard, 1890	4.3	3.3	1.4
Trinema enchelys (Ehrenberg, 1838) Leidy, 1878	8.7	4.8	2.7
Trinema lineare Penard, 1890	19.2	14.0	27.4

539