

1 **Protist diversity on a nature reserve in NW England – with particular reference to their role in soil**
2 **biogenic silicon pools**

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11 **ABSTRACT**

12 Soil protists play fundamental roles in many earth system processes, yet we are only beginning to
13 understand the true diversity of the organisms involved. In this study we used conventional
14 (microscopy-based) methods to characterise the diversity and estimate protist population sizes in soils
15 from a variety of distinct habitats within Mere Sands Wood nature reserve in NW England. We
16 produced population size data for over ninety soil protists belonging to two major eukaryotic
17 functional groups: testate amoebae (TA) and diatoms, adding substantial ‘cryptic diversity’ to the
18 nature reserves recorded biota. From these population size data we estimated relative contributions
19 of TA and diatoms to soil biogenic silicon (BSi) pools and found significant correlations between taxon
20 richness and the TA and diatom Si pool. This could indicate that protist functional diversity can
21 influence terrestrial BSi pools, especially in early successional plant communities where TA and
22 diatoms can potentially increase Si mineralisation and/or create Si ‘hot spots’ and hence, the biological
23 availability of this element for subsequent plant uptake. TA were particularly abundant in mor humus
24 type soils further supporting the idea that they could be important players in nutrient cycling in such
25 soils. Overall, we demonstrate this is a useful approach in order to start to attempt to estimate the
26 role of protists in the Si cycle and other ecological processes.

27 **KEYWORDS:** protist, population size, diversity, testate amoebae, diatoms, biogenic silicon

28 **1. Introduction.**

29 Over the last 40 years there has been a profound shift in the way in which many scientists view the
30 role of life in the context of the whole Earth – from life being viewed as just inhabiting the planet, to
31 it playing major roles in the workings of the Earth System. This systems view of the Earth mainly
32 developed during the 1980’s and 90’s and was influenced by several sources; including the extra-
33 planetary perspective that came from NASA’s Apollo Missions and the Gaia hypothesis developed by
34 James Lovelock and Lynn Margulis in the early 1970’s (Hamilton and Grinevald, 2015). From the start
35 what was to become Earth Systems science – especially as envisaged by the Gaia hypothesis put a
36 strong emphasis on the role of microorganisms in the Earth system (Margulis and Lovelock, 1974).
37 Lovelock (1993) also argued that soils made an interesting model for the planet as a whole, because
38 life plays a major role in constructing soils rather than merely adapting to abiotic soil conditions.

1 Indeed the development of soils and the associated vegetation cover may have played significant roles
2 in Earth history through the climatic effects of the weathering of soil minerals on the global climate
3 (Schwartzman and Volk, 1989; Lenton et al., 2012). However, while clearly of crucial importance, the
4 ecology of soils in general, and soil microbes in particular, was until recently somewhat understudied.
5 As one of us wrote a few years ago: ‘The soil is largely ‘out of sight’ to an ecologist without a spade
6 and, for much of the 20th century, this has meant that it was also ‘out of mind’ to most ecologists. At
7 best, it tended to be treated as a black box, with the behaviour of its inhabitants lumped together
8 under simple labels such as decomposers or nitrogen fixers’ (Wilkinson, 2008, p596). Even within
9 microbial ecology soil protists have been far less studied than bacteria or fungi – although there are
10 some signs that this is starting to change (Wilkinson et al., 2012). In some recent studies both
11 traditional approaches (Finlay and Fenchel, 2001; Domonell et al., 2013; Geisen et al., 2014; Andriuzzi
12 et al., 2016) and modern (Geisen et al., 2015; Lara et al., 2015) sequence-based analyses have been
13 applied to study natural protist communities in soils.

14 From before the full development of the current systems approach to global ecology it was known
15 that life was sustained by the cycling of energy and chemical elements (e.g. Hutchinson, 1970),
16 however a systems approach can be seen as putting particular emphasis on the merging of organismal
17 and ecological physiology (Wilkinson, 2003; 2006). To understand such processes we need to be able
18 to quantify microbial diversity, population sizes and biomass. Given that, as Sean Nee (2004, p 804)
19 emphasised, ‘the contribution of visible life to biodiversity is very small indeed’ this is no small task –
20 much of the life we need to quantify to understand the world is microscopic. This vast expanse of
21 microscopic biological diversity forms part of what has been called ‘cryptic diversity’ and is not
22 included in most conservation surveys (Esteban and Finlay, 2010). Indeed the microbial aspect of this
23 cryptic diversity is seldom assessed in biodiversity surveys – it’s unusual to have good data on even
24 the small metazoan fauna and fungi (e.g. Corbet, 2011). One substantial and understudied aspect of
25 this cryptic diversity in soils is composed of protists (Geisen et al., 2015). A number of papers from the
26 1950’s to the 1970’s provided some limited estimates of Testate amoebae production in soils –
27 especially the work by Bonnet and Thomas (1955); Lousier and Parkinson (1984) and Schönborn (1965,
28 1978, 1983), this work is extensively summarised and evaluated by Wilkinson and Mitchell (2010). As
29 Heger et al. (2014) have argued, there needs to be a resurgence in field research on microbial
30 eukaryotes because this area of study is currently limited by the shortage of data on natural protist
31 communities – especially heterotrophic protists.

32 In this study we constrain the problem to manageable proportions by considering just two functional
33 groups of protists – testate amoebae (TA) and diatoms, and use the silicon (Si) cycle as an example of
34 the potential importance of soil protists. Various pro- and eukaryotes are evolutionarily adapted to
35 synthesize siliceous structures (biosilicification). These organisms use monomeric silicic acid (H_4SiO_4)
36 for synthesis of biogenic silica, i.e. hydrated amorphous silica ($SiO_2 \cdot nH_2O$) (e.g. Ehrlich et al., 2010). In
37 terrestrial ecosystems the accumulation of biogenic silica of microbial (bacteria, fungi), phytogenic
38 (plants), protophytic (diatoms), protozoic (TA) and zoogenic (sponges) origin results in formation of
39 corresponding biogenic Si (BSi) pools (Puppe et al., 2015; Sommer et al., 2006). TA form a polyphyletic
40 group traditionally placed in the phylum Rhizopoda (Margulis and Chapman, 2009) but now split
41 between two Eukaryote supergroups, the Amoebozoa (for the Arcellinida) and SAR (for the Euglyphida
42 and other taxa with filose pseudopodia) (Adl et al, 2012). The members of the Euglyphida make self-
43 secreted siliceous plates (idiosomes) (Ogden and Hedley, 1980). The diatoms also form part of the SAR
44 eukaryotic supergroup forming the Diatomea (classically the Bacillariophyta) in the classification of

1 Adl et al. (2012). These are predominately marine or freshwater photosynthetic microbes with tests
2 (frustules) formed from amorphous silica and so require dissolved Si for growth (Margulis and
3 Chapman, 2009). Although primarily aquatic, in some situations they may be important for retaining
4 BSi in soils and peats (Kokfelt et al, 2009; Alfredsson et al., 2015). Accumulation and recycling of BSi in
5 terrestrial ecosystems influence fluxes of dissolved Si from the continents to the oceans, thus act as a
6 filter in the global Si cycle (Dürr et al., 2011; Struyf and Conley, 2012). Although this biogenic control
7 mechanism has been generally recognized for decades (e.g. Bartoli, 1983; Meunier et al., 1999; Struyf
8 and Conley, 2012) quantitative information on particular terrestrial BSi pools are surprisingly rare
9 (Sommer et al., 2006). We hypothesise that diversity of TA and diatoms differs within spatially
10 heterogenic terrestrial ecosystems (i.e. various habitats within a single nature reserve) and this, in
11 turn, leads to corresponding patterns in the protophytic and protozoic Si pools. The aims of our work
12 were: 1) to quantify soil protist diversity and population sizes in various habitats and 2) to investigate
13 relationships between soil protist diversity and corresponding protophytic and protozoic Si pools.

14 **2. Methods**

15 2.1 *Study site and sampling procedure*

16 Mere Sands Wood (MSW) Nature Reserve (53°63'55" N, 2°83'71" W) is an artificial, post sand-mined
17 catchment situated in the county of Lancashire in North West England. The soils of MSW have an
18 interesting geological history and provide the best sections available for understanding the deposition
19 of the Shirdley Hill Sands of Lancashire laid down during the late glacial and early post glacial periods
20 (Chiverell et al., 2004). Sand extractors recognised the commercial value in the glass making industry
21 and between 1974 and 1982 the site was quarried. Extracted areas were then landscaped into shallow-
22 edged lakes. This spatially heterogeneous site is now managed as a nature reserve and is characterised
23 by a series of artificially created lakes surrounded by deciduous and coniferous woodland with smaller
24 patches of grassland, heathland and scrub. This is the first study investigating soil protists on the
25 nature reserve. Field sampling was undertaken over two consecutive days in September 2011 and
26 directed towards capturing the range of environmental variability within terrestrial habitats on the 42
27 hectare reserve. Four broad habitat types were identified: (DW) early successional deciduous
28 woodland comprised open Silver birch (*Betula pendula*) canopy with a ground cover of grasses
29 (Poaceae spp.) and bryophytes on a sandy substrate, (CW) artificially planted mature Scots pine (*Pinus*
30 *sylvestris*) woodland with ground cover comprised of male fern (*Dryopteris* spp.) and bramble (*Rubus*
31 *fruticosus* agg.), (G) Mesotrophic grassland mainly comprised of grasses (Poaceae spp.) and Rushes
32 (*Juncus* spp.) periodically grazed by sheep and (B) low silt island in the centre of a shallow lake used
33 by roosting waterfowl and covered by bryophytes and Poaceae spp. A randomly placed quadrat
34 (1x1 m) was placed within each habitat type and three replicate subsamples were taken at random
35 from within it. Each sample comprised about 20 g of leaf litter (0 – 2.5 cm) and an equivalent amount
36 from the soil horizon directly below the leaf litter horizon (2.5 – 5 cm). All samples were frozen prior
37 to laboratory analysis.

38 2.2 *Slide preparation and protist counting*

39 2.2.1 *Diatoms*

40 A known weight of sample was oven-dried at 105 °C for 48 hours. The range of sample used varied
41 between 0.1 g for leaf litter to 1 g for samples with high mineral content. Diatom samples require

1 digestion to remove organic matter. In this study diatom frustules were treated with 50 ml of 30 %
2 hydrogen peroxide (H₂O₂) to remove organic matter. Hydrochloric acid (10 % HCL) was added to
3 remove calcium carbonates and the supernatant was removed after >12 hours of sedimentation.
4 Samples were re-suspended and cleaned in 30 ml de-ionised water and a 0.3 ml aliquot of the diatom
5 suspension was dried on a glass cover slip and the clean diatom valves were mounted in Naphrax.
6 Diatoms were identified to species level and abundance was measured as the number of individuals
7 per unit dry matter. Diatom frustules were counted under oil immersion at 1000 x magnification using
8 a Meiji Techno microscope. Where possible, a minimum of 400 frustules were counted and identified
9 when more than half the valve was intact. As a result of the differences between aquatic and soil
10 diatoms (Schuttler 1986, Lund 1946), the taxonomy of soil diatoms mainly followed that of Lund
11 (1946). Micrographs illustrating how we defined the various morpho-species can be found in the
12 Appendix of Creevy (2013).

13 2.2.2 *Testate amoebae*

14
15 To extract TA a known weight (ranging between 0.1 g for leaf litter to 1 g for samples with high mineral
16 content) of oven-dried (50 °C) sample was soaked and disaggregated in 50 ml de-ionised water and
17 stirred occasionally. The material was washed through a sieve (250 µm) to remove plant and coarse
18 particulate matter. The material was washed into a 50 ml centrifuge tube and centrifuged at 3000 rpm
19 for three minutes and the supernatant was discarded. Samples were stained with Rose Bengal in order
20 to allow the identification of dead and live individuals. TA were identified to species level and
21 abundance was measured as the number of individuals per unit dry matter. Amoeba tests were
22 identified and counted at 200 x and 400 x magnification using a Meiji Techno microscope. Search effort
23 was restricted to 150 individuals per sample (following Davis and Wilkinson, 2004; Payne and Mitchell,
24 2009; Booth et al., 2010). Morphological identification of TA is based on the guides of Charman et al.
25 (2000), Clarke (2003), Scott et al. (2001), Ogden and Hedley (1980) and Mazei and Tsyganov (2006) as
26 well as some identification keys (Mitchell, unpublished). For some samples SEM (Scanning Electron
27 Microscope) pictures were taken to confirm the identification. For this 0.2 ml of the same solution
28 used for optical microscopy was placed onto aluminium stubs and the samples were air-dried in a
29 desiccator for 3 days prior to SEM analysis. As with the diatoms, described above, micrographs of
30 many taxa – illustrating how the morpho-species were interpreted – can be found in the Appendix of
31 Creevy (2013) available online from Liverpool John Moores University.

32

33 2.3 *Quantification of protophytic and protozoic Si pools*

34

35 Protophytic Si pools represented by diatoms were quantified by multiplying the density of biogenic
36 silica (2.35 g cm⁻³) with corresponding volumes (µm³) of silicified frustules (see, e.g., Conley et al.,
37 1989). In order to avoid very time-consuming volume measurements of every single diatom, diatom
38 volumes (µm³) and lengths (µm) of a sample of 45 pennate diatoms were measured using a laser
39 scanning microscope (Keyence VK-X110, magnification 200-2.000 x) at ZALF in Müncheberg
40 (Germany). For laser scanning microscopy diatoms were taken from soil suspensions by
41 micromanipulation, washed in distilled water and placed on clean object slides. After air drying images
42 of diatoms were acquired and analysed. Details on laser scanning microscopy can be found in Puppe
43 et al. (2016). Several size categories represented by the length of pennate individuals (smallest size
44 category: 21-25 µm, biggest size category: 96-100 µm) of diatoms were determined and corresponding

1 mean volumes per size category were calculated using an exponential function. Subsequently, this
2 allowed only the length of pennate diatoms to be measured, so a relatively fast scanning of soil
3 suspensions under the light microscope was possible. Due to the fact that diatoms were air dried for
4 analyses under the laser scanning microscope only total diatom but no frustule volumes could be
5 measured because entire frustule dimensions (i.e. frustule thickness and shape) were not visible in
6 most cases. This is why corresponding silica contents were calculated assuming that only 10 %
7 (conservative estimate, see, e.g., Sicko-Goad et al., 1984) of the complete diatom volume represent
8 the frustule. As biogenic siliceous structures generally consist of hydrated amorphous silica
9 ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$), an average water content of about 10 % was assumed to avoid an overestimation of
10 protophytic Si pools (see Mortlock and Froelich, 1989). Protozoic Si pools represented by euglyphid
11 TA were quantified by multiplication of known silica contents per shell of diverse taxa (Aoki et al.,
12 2007) with corresponding individual numbers (living plus dead TA, for details see Puppe et al., 2014,
13 2015). Silica ($M = 60.08 \text{ g mol}^{-1}$) contents of diatom frustules and TA shells were converted to Si ($M =$
14 $28.085 \text{ g mol}^{-1}$) contents by multiplication with 28/60. In a final step protophytic and protozoic Si pools
15 were converted to dry mass (dm) and given as concentration ($\text{ng Si g}^{-1} \text{ dm}$).

16

17 2.4 Numerical analysis

18 The Shannon's diversity Index (SDI) (Shannon, 1948) was used to examine the microbial diversity of
19 the taxa found in each sample and diversity statistics were obtained using PAST software (Hammer et
20 al., 2001). Formal normality tests in such a context is not recommended (although widely used) and
21 has very low power for small (i.e. <50) sample sizes (Ruxton et al., 2015). Therefore, we have been
22 conservative and mainly used non-parametric Mann-Whitney U test and Kruskal-Wallis test to
23 investigate differences in taxon richness, Shannon's diversity and abundance of TA and diatoms
24 between different habitats and also between leaf litter and soil. Using the General Linear Model (GLM)
25 for One-way Analysis of Variance (ANOVA) we found TA taxon richness and Shannon's index data were
26 normally distributed and met the assumptions for homogeneity of variance, therefore, we used
27 parametric tests including Tukey's post-hoc tests to investigate differences in diversity indices
28 between habitats. Spearman's correlation (r_s) was used to test relationships in taxon richness and BSi
29 pools between habitats and through the vertical soil profile (i.e. litter and soil). Statistical analyses
30 were performed using SPSS (version 22.0 IBM Corp.).

31 3. Results

32 A total of 93 soil protists from two functional groups were identified in leaf litter and soils from a range
33 of habitats across the nature reserve. These comprised of 41 TA (19 Euglyphida and 22 Arcellinida)
34 and 52 diatom taxa. Despite the higher taxon richness in diatoms (many of which were aquatic forms
35 found in bryophytes and soils on an island within a shallow lake), the overall relative abundance of TA
36 individuals was higher (59.3 %) compared with diatoms (40.7 %). With the exception of bryophytes,
37 there was a general trend of higher TA taxon richness in litter and soil substrates compared with
38 diatoms across all terrestrial habitats (Fig. 1a and b). The median testate taxon richness in litter and
39 soil ($n = 24$) was 12.0 with interquartile ranges of 9.5 to 15.0 taxa compared to diatoms ($n = 24$) median
40 taxon richness of 2.0 with interquartile ranges of 0 to 11 taxa. Median Shannon's diversity was also
41 higher for testates (Index = 2.0) with interquartile ranges of 1.8 to 2.2 compared to diatoms (Index =
42 0.6) with interquartile ranges of 0 to 2.1. There were statistically significant differences found between
43 TA and diatom taxon richness ($U = 127.5000$, $p < 0.001$) and Shannon's diversity ($U = 144.000$, $p =$

1 0.003). TA were detected in all leaf litter and soil samples in contrast with diatoms which were not
2 detected in litter and soils associated with acidic, mor humus soils, specifically, those associated with
3 mature *P. sylvestris* woodland.

4 [Figures 1a and b]

5

6 3.1 *Community composition*

7 3.1.1 *Diatoms*

8 The most abundant taxa were: *Navicula seminulum* (20.5 %), *Hantzschia amphioxys* (7.9 %),
9 *Achnanthes minutissima* (7.0 %) and *Navicula minima* (6.9 %). The median diatom taxon richness was
10 2.0 with interquartile ranges of 0 to 11.0 with the minimum and maximum number of taxa per sample
11 ranging from an absence of diatom taxa in coniferous woodland to thirty one taxa in litter and soils
12 associated with bryophytes on an island within a shallow lake. Excluding bryophytes, the median taxon
13 richness in deciduous woodland, coniferous woodland and grassland habitats collectively was 1.0 with
14 interquartile ranges of 0 to 4.25. Median Shannon's diversity was 0.6 with interquartile ranges from 0
15 to 2.1 and ranged between 2.5 (interquartile range 0.5) in bryophytes to 0.28 (interquartile range
16 0.84) in deciduous woodland. There were statistically significant differences in taxon richness ($X^2 (2) =$
17 $19.335, p < 0.001$) and Shannon's diversity ($X^2 (2) = 17.434, p < 0.001$) between the four habitats
18 studied.

19 3.1.2 *Testate amoebae*

20 The most abundant taxa were: *Trinema enchelys* (18.3 %), *Trinema lineare* (13.1 %), *Euglypha rotunda*
21 (12.6 %), *Trinema complanatum* (7.6 %) and *Euglypha tuberculata* type (6.4 %). The median testate
22 taxon richness was 12.0 with interquartile ranges of 9.5 to 15.0 with the minimum and maximum
23 number of taxa per sample ranging from four taxa in grassland soils to seventeen taxa found in soil
24 associated with bryophytes on an island within a shallow lake respectively. Median Shannon's diversity
25 was 2.0 with interquartile ranges of 1.8 to 2.2 and ranged from 2.2 (interquartile range 0.4) in
26 coniferous woodland to 1.8 (interquartile range 0.3) in grassland. ANOVA was used to test for
27 differences in mean taxon richness and Shannon's diversity index between the four habitats studied
28 and there were statistically significant difference in taxon richness ($F_{3,20} = 6.242, p = 0.004$) and
29 Shannon's diversity index ($F_{3,20} = 3.526, p = 0.034$) between the four habitats. Tukey's post hoc tests
30 revealed that grassland diversity indices were significantly different from other habitats studied ($p <$
31 0.05).

32 3.2 *Protophytic and protozoic Si pools*

33 Diatom volumes were significantly correlated with diatom lengths ($r_s = 0.868, p < 0.001$) showing a
34 strong exponential relationship. Calculated Si contents of diatom frustules ranged between 127 pg
35 and 1,876 pg for smallest (21-25 μm) and biggest (96-100 μm) diatom size categories, respectively
36 (data not shown). Protophytic Si pools (Fig. 2a) were significantly smaller in both litter and soil
37 substrates compared with protozoic Si pools (Fig. 2b) ($U = 74.000, p < 0.001$). Protozoic Si pool was
38 greatest in leaf litter and soils associated with early successional deciduous woodland (relative
39 proportion 34.2 %) compared with other habitats studied. There was no difference in protozoic Si
40 pools between habitats ($X^2 (3) = 6.687, p = 0.083$). In contrast, there was a significant difference in

1 protophytic Si pools between habitats ($\chi^2 (3) = 19.861, p < 0.001$). There was a significant positive
2 correlation between euglyphid taxon richness and protozoic Si pool ($r_s = 0.668, p < 0.001$). The
3 relationship between diatom taxon richness and protophytic Si pool followed a similar pattern with a
4 stronger positive correlation ($r_s = 0.988, p < 0.001$) (Figs. 3a and b). Overall, we found no statistical
5 difference in protist Si pools in the leaf litter compared with the soil horizon. Protophytic Si pool was
6 higher in soils ($18.0 \pm 7.0 \text{ ng Si g}^{-1} \text{ dm}$) compared with the organic leaf litter horizon ($9.8 \pm 5.5 \text{ ng Si g}^{-1}$
7 dm), although the high standard errors indicate considerable variability in diatom Si pools in the
8 vertical soil profile. The trend was reversed for testates, we found protozoic Si pool highest in the
9 organic leaf litter horizon ($63.2 \pm 6.3 \text{ ng Si g}^{-1} \text{ dm}$) compared with the soil horizon ($44.2 \pm 8.7 \text{ ng Si g}^{-1}$
10 dm) with high variability in BSi pools between samples. In terms of overall BSi pools, the most
11 important diatom taxon was *Hantzschia amphioxys*, accounting for a substantial proportion (46.3 %) of
12 the total protophytic Si pool, while *Trinema enchelys* and *Trinema lineare* were the most important
13 taxa in the protozoic Si pool with proportions of 23.9% and 16.2 %, respectively. The mean relative
14 contributions of different taxa to the overall protophytic and protozoic Si pool in the various habitats
15 is provided (Table 1).

16 Figures 2a and b

17 Figures 3a and b

18

19 **4. Discussion**

20 Grime (1998) emphasised that often it is the taxa with the greatest biomass that drive key ecosystem
21 processes, and the same argument can be made for the whole Earth system (Wilkinson, 2006). Soils
22 harbour a copious abundance and diversity of protists across all known eukaryotic supergroups
23 (Geisen et al., 2015) and better understanding of their distribution in various habitats will lead to a
24 better understanding of their functional roles in different soil systems. In this study we identified over
25 ninety soil protists belonging to two major eukaryotic functional groups, this adds substantial cryptic
26 diversity (sensu Esteban and Finlay, 2010) to the reserves biota. We show the inclusion of just two
27 groups of protists into a biodiversity survey on a single nature reserve has led to a better
28 understanding of the potential of terrestrial diatoms and TA to create Si hotspots in soil. The
29 importance of including soil biodiversity as a criterion in management of conservation land for human
30 health was recently highlighted by Wall et al. (2015) and this is especially relevant to Mere Sands
31 Wood nature reserve which is surrounded by intensive arable agriculture which likely has an
32 impoverished soil microbial community. Recent studies (for example, Lara et al., 2015; Geisen et al.,
33 2015) have shown that the use of modern molecular approaches can be extremely powerful and
34 effective methods to recognise and quantify soil protists and can be used in conjunction with
35 morphology-based taxonomy approaches. Our preliminary results are in agreement with comparative
36 molecular data (Geisen et al., 2015) suggesting Amoebozoa are perhaps more important in grassland
37 and forest soils compared with Diatoms (Stramenopiles). The combination of an inter-disciplinary
38 approach along with the inclusion of microorganisms and microscopic metazoan fauna into
39 biodiversity surveys will almost certainly lead to a better, more comprehensive understanding of
40 microbially driven ecosystem processes. In interpreting our data it should be remembered that this is
41 a snapshot of protist diversity at just one time in the year (September) and that protist numbers vary
42 throughout the year (Couteaux, 1976; Mitchell et al., 2008).

1 Very few studies have quantified both TA and diatoms collectively in the soil environment. To our
2 knowledge there is only one study which assessed the usefulness of both groups for biomonitoring in
3 agricultural soils and found them sensitive to different farming practices (Heger et al., 2012).
4 Considering that diatoms and some testates both utilize Si for shell construction, we might expect the
5 diversity and abundance of one group to follow that of the other but the results of this study do not
6 support this idea. Overall, we found that TA were more abundant in leaf litter and soils compared with
7 diatoms. TA were identified in all habitats, whereas diatoms were not identified in leaf litter and soils
8 associated with mature coniferous woodland. This is perhaps not surprising as light levels are low on
9 this woodland floor and light is usually considered a prerequisite for diatom production, although
10 mixotrophy has occasionally been described in freshwater ecosystems (Bavestrello et al., 2000). It is
11 recognised that some species of TA harbour photosynthetic symbionts and these mixotrophic taxa are
12 especially important in nutrient-poor environments (Gomaa et al., 2014), but there are no records of
13 photosynthetic endosymbionts in euglyphid TA, suggesting that light may not be a factor for these
14 testates. The results are in agreement with Wilkinson and Mitchell's (2010) suggestion that TA have a
15 particularly important role in nutrient cycling in mor humus type soils.

16 Results from microcosm and field studies indicate that microbial species diversity, functional group
17 diversity and community composition can all influence ecosystem process rates (McGuire and
18 Treseder, 2010). In the present study we found strong relationships between protist taxon richness
19 and soil BSi pools. This result indicates that protist diversity and community composition can influence
20 terrestrial BSi pools and provides a better understanding of the eukaryotic microbial mechanisms
21 involved in Si cycling in terrestrial environments (Opalinka and Cowling, 2015; Alfredsson et al., 2015).
22 The species richness-productivity relationship (SRPR) has been extensively studied in plants (Šímová
23 et al., 2013), and to a lesser extent in prokaryotes (Zhalnina et al., 2015), but far fewer studies consider
24 the eukaryotic SRPR relationship. In terms of run-off of terrestrial Si into waterbodies, Wilkinson and
25 Mitchell (2010) suggest that testates with siliceous idiosomes (such as *Euglypha* spp.) could potentially
26 have increased the rate of Si mineralization in soils with direct implications for nutrient availability for
27 diatoms in aquatic ecosystems. To better understand future effects of human-induced land-use
28 change on ecosystem processes, such as the Si cycle (see, e.g., Vandevenne et al., 2015), we need to
29 better understand the microbes involved and where Si is accumulated in the landscape (Sommer et
30 al., 2006). As (alkaline) extraction methods (an overview is given in Sauer et al. 2006) are hardly able
31 to differentiate between various BSi pools, direct (microscopical) methods are needed for a more
32 accurate quantification of single (e.g. phytogenic, protophytic, protozoic) BSi pools. Furthermore, we
33 show that Si-contents are species-related (or at least size-related), so it is necessary to get information
34 on population composition. Here we demonstrate that population size data provides a mechanism for
35 understanding protist functional roles in natural soils. Future research should focus on the
36 mechanisms by which microbial diversity influences Si cycling in terrestrial environments, in particular,
37 the rate of Si turnover in testates and diatoms to determine the relative importance of protists to the
38 Si pool.

39
40 This morphology-based approach which characterises protozoic and protophytic population sizes and
41 calculates corresponding BSi pools provides one example of how we can differentiate between
42 different BSi pools in soil systems (Sommer et al., 2006). However, we do acknowledge that our results
43 provide an indirect measure of protozoic and protophytic soil Si pools and do not take into account
44 accumulation, redistribution and losses and gains of Si during soil development. In this study we

1 compared BSi pools represented by euglyphid TA and diatoms and recent molecular work (Lahr et al.,
2 2015) suggests the protist Si pools calculated here could be underestimated as some arcellid TA also
3 produce Si rich shells. Interestingly, we found a similar trend in BSi pools and taxon richness across
4 different habitats. In particular, higher taxon richness in testates and diatoms were observed in early
5 successional vegetation (i.e. bryophytes and early successional deciduous woodland) and this
6 corresponded to greater BSi pools, potentially creating more Si hot spots in these soils. The results of
7 Puppe et al. (2014) showed that the protozoic Si pool is built up rapidly at very young (< 10 yrs) initial
8 ecosystem states and is strongly linked to plant growth. In soils of forested ecosystems protozoic Si
9 pools were larger compared to the ones in initial ecosystems and could reach up to 5 kg Si ha⁻¹ (Puppe
10 et al., 2015). Annual biosilicification rates of living TA (17–80 kg Si ha⁻¹) were comparable to or even
11 exceeded reported data of annual Si uptake by trees (Puppe et al., 2015; Sommer et al., 2013). As this
12 study was originally conceived to estimate taxon diversity (rather than focusing on the Si cycle) we did
13 not determine bulk densities and so are unable to calculate BSi pools per hectare or square meter -
14 for example, such data can be found in Puppe et al. (2014, 2015) or Sommer et al. (2013).

15

16 Compared with protozoic Si pools, fewer studies consider the role of protophytic Si pools in terrestrial
17 environments, although the potential of diatoms for Si cycling, especially in wetlands, has been
18 recognised (Clarke, 2003; Kokfelt et al., 2009; Struyf and Conley, 2009; Struyf et al., 2010). Aoki et al.
19 (2007) demonstrated the potential importance of amoebal Si pools to Si cycling in terrestrial
20 environments because of their small-size, rapid turnover time and sheer abundance in soil. In this
21 study, the larger soil diatoms, specifically *Hantzschia amphioxys* tended to have the greatest
22 proportion of the protophytic Si pools. In contrast, the highest proportion of BSi pools in soil testates
23 were found amongst the small, bacterivorous, pioneer taxa, for instance, *Trinema enchelys* and
24 *Trinema lineare*, which collectively made up a significant proportion (40.1 %) of the total protozoic Si
25 pool. Diatoms take up dissolved Si, convert it to biogenic forms for frustule formation, with Si being
26 mobilized again upon decomposition (Opalinska and Cowling, 2015). Therefore, as with TA, they are
27 increasing Si mineralisation and hence, the biological availability of this element for subsequent plant
28 uptake (Wilkinson, 2008).

29 Overall, we found no statistical difference in protist Si pools in the leaf litter compared with the soil
30 horizon. Alfredsson et al. (2015) found that soil diatoms were important components of soil
31 amorphous silica storage in the top organic horizon of peatlands and less so in the mineral horizons
32 of shrub tundra. In this study we found diatom Si pools were higher in soils compared with the organic
33 leaf litter horizon which may be due to several compounding and auto correlating factors, for example,
34 1) less light in the pine dominated organic, acidic soils, 2) greater year round interstitial moisture
35 content and 3) greater nutrient availability in the mineral soils, thus promoting greater diatom
36 productivity and hence Si uptake. The high standard errors indicate considerable variability in diatom
37 Si pools in the vertical soil profile. The trend was reversed for TA, where we found euglyphid Si pools
38 highest in the organic leaf litter horizon. For grassland, low standard errors suggest that these taxa are
39 distributed in a relatively uniform manner through these soils. In contrast, for coniferous and
40 deciduous woodlands large standard errors indicate a more patchy distribution and suggest that
41 further research is needed to explain the variability in protist Si pools. Opalinska and Cowling (2015)
42 also found large variations in BSi pools in coniferous catchments suggesting pH is a very important
43 factor in altering BSi dissolution, for instance, once you start to get alkaline soils Si becomes much
44 more soluble and available. We hypothesize that hot spots of Si from decaying TA shells etc. may be

1 more valuable to plants in acidic soils when Si is less available from parent material. Importantly, if we
2 are to improve the development of Si cycling models (Opalinska and Cowling, 2015), it is essential that
3 we quantify protist Si pools and incorporate this data into existing models because of the potential for
4 these microorganisms to create Si hot spots in the soil.

5 **5. Conclusion**

6 We conclude that quantification of microbial diversity in soils is vital in order to begin to estimate the
7 full diversity of life on a nature reserve or other location. However, species lists are not enough – in
8 order to start to understand the functioning of microbes in the Earth system we need to quantify
9 population sizes. Applying such data to questions on soil BSi we found that protophytic and protozoic
10 Si pools might be significant for creating BSi pools/hot spots, particularly in initial ecosystem states
11 and are linked especially to vegetation. Further research into the eukaryotic SRPR is needed to better
12 understand links between microbial diversity and ecosystem function. As our provisional calculations
13 for BSi pools illustrate, once population data on microbial taxa are available it starts to be possible to
14 attempt to quantify their role in various ecological processes such as the Si cycle.

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