

**Tree encroachment may lead to functionally-significant changes in peatland testate amoeba communities**

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**ABSTRACT**

Climate change is likely to cause increased tree recruitment on open peatlands but we currently have little idea what consequences this vegetation change may have below-ground. Here we use transects across forested to open bog ecotones at three Russian peatland complexes to assess potential changes in the most abundant group of peatland protists – the testate amoebae. We show that the testate amoeba communities of forested and open bog are markedly different with a very abrupt boundary at, or near, the vegetation ecotone. Changes along our transects suggest that tree encroachment may reduce the trophic level of testate amoeba communities and reduce the contribution of mixotrophic testate amoebae to primary production. Our study strongly suggests that increased tree recruitment on open peatlands will have important consequences for both microbial biodiversity and microbially-mediated ecosystem processes.

**KEYWORDS:** Peatland; Forest; Tree; Protist; Climate; Carbon

Climate change is causing treelines to move pole-wards and to higher altitudes around the world (Harsch et al., 2009). Peatland ecosystems are a globally-significant carbon store (c.4-600 GtC (Yu,

2012; Loisel et al., 2014)) with two alternative stable states as unforested 'open' bogs and forested bogs (often known by their Russian name 'ryam')(Agnew et al., 1993). Modelling and experimental evidence show that climatic warming and water table drawdown can lead to a switch between these states with the establishment of trees on formerly-open peatland (Heijmans et al., 2013; Limpens et al., 2014; Holmgren et al., 2015). It is reasonable to expect that rising temperatures and increased drought frequency, combined with ongoing anthropogenic drainage, will lead to increasing areas of boreal peatland switching to tree domination. There is both palaeoecological evidence for greater forest cover in peatland-dominated boreal regions during warmer phases of the Holocene (MacDonald et al., 2008) and monitoring data suggesting recent increases in tree and shrub recruitment in many peatland regions (Esper and Schweingruber, 2004; Shiyatov et al., 2005; Berg et al., 2009). The incursion of trees into previously open bogs has the potential to create positive feedback loops through increased transpiration, interception and shade leading to drying of the bog surface (Waddington et al., 2015). The consequences for microbial communities of such a switch in vegetation are largely unknown but may have important implications for carbon and nutrient cycling. Here we use a space-for-time substitution approach (Blois et al., 2013) to assess possible changes in communities of the key eukaryotic microbes of peatlands – the testate amoebae – with tree establishment. Testate amoebae are the most abundant protists in peatlands (Gilbert et al., 1998) and play important roles in carbon and nutrient cycling (Wilkinson and Mitchell, 2010).

We positioned ten transects of 30-45m length across the forested to open bog ecotone at three peatland complexes in western Siberia (Mukhrino 60.9°N, 68.7°E), the Karelia region of northwest Russia ('Black River' 66.5°N, 32.9°E) and the Penza region of European Russia (Morsovo 53.8°N, 42.3°E). Transects (3-4 per site) were labelled based on a qualitative judgement of the centre point of the vegetation ecotone (0m), with positive numbers representing the open bog and negative numbers the forested bog ends of the transect respectively. Samples were extracted every 5m by removing the upper 5cm of bryophytes and any litter (c. 25cm<sup>3</sup>), giving a total of 74 samples across all sites. Testate amoebae were extracted, identified and enumerated under the microscope using standard methods (Booth et al., 2010)(Supplementary Table 1). The mean count total was 211 tests per sample and the minimum was 100, sufficient to adequately characterise the community (Payne and Mitchell, 2009). To assess community change along the transects we summarised the datasets using first axis scores from an NMDS ordination on Bray-Curtis dissimilarity (Bray and Curtis, 1957). Results (Fig. 1B) show large changes in most transects with a very abrupt shift between low and high axis scores. To identify points along the transects which maximised community difference we applied non-parametric change-point analysis (nCPA; (Qian et al., 2003)) (Fig. 1A). Identified change-points were all immediately adjacent to the centre-point of the vegetation ecotone as judged in the field, with two exceptions from the Morsovo site where the vegetation change is more gradual. Redundancy analysis (accounting for the transect structure) showed a highly significant difference between the amoeba communities either side of these points (Hellinger-transformed data; 17.8% variance, P=0.001). This difference remained highly significant when accounting for water table depth (measured in Mukhrino only; 7.5% variance, P=0.002) and more variance was explained by the difference across the change points than by tree stem density for the only site where this was recorded (Mukhrino only; 29.9% variance, P=0.001 versus 19.2% variance, P=0.001).

We used Indicator Value analysis (IndVal (Dufrêne and Legendre, 1997)) to identify taxa typifying samples on either side of the change points. These groups have clear differences (Fig. 1A). Taxa

78 typifying the open bog end of the transects are generally larger and mixotrophic (e.g. *Hyalosphenia*  
79 *papilio*; *Archerella flavum*) while taxa typifying the forested end are often smaller and bacterivorous  
80 (e.g. *Assulina muscorum*; *Corythion dubium*). We used Bray-Curtis dissimilarity to assess pairwise  
81 mean similarity to communities of mineral soil forests (111 analyses from across Siberia (Malysheva,  
82 2011)) and open peatlands (68 analyses (Lamentowicz et al., 2015b)). These results showed some  
83 variability but communities at the open bog end of the transects showed greater similarity to other  
84 analyses from open peatland and differed strongly ( $p < 0.01$ ; Supplementary Fig. 1) from communities  
85 at the forested end, which were more similar to mineral soil forest.

86 To assess the possible functional significance of these changes we considered two key functional  
87 traits of testate amoeba communities: the proportion of mixotrophic taxa and the community-  
88 weighted mean aperture diameter, which is increasingly used as a metric of trophic position  
89 (Fournier et al., 2012; Lamentowicz et al., 2013; Fournier et al., 2015; Lamentowicz et al., 2015a).  
90 Aperture diameter data were taken from the literature and direct observations and assigned to five  
91 size classes (1=0-10 $\mu$ m; 2=10-20 $\mu$ m; 3=20-30 $\mu$ m; 4=30-40 $\mu$ m; 5=>40 $\mu$ m) using a mean of the  
92 maximum and minimum dimensions where reported. Results show an abrupt increase in the  
93 proportion of mixotrophs from forested to open bog (although mixotrophs were rare in Morsovo).  
94 Testate amoebae from the open bog end of the transects generally had larger apertures, suggesting  
95 larger prey and higher trophic position. Both these differences were highly significant ( $P < 0.01$ ; Fig.  
96 1C & Fig. 1D).

97 Differences in testate amoeba communities between forested and open bog are not particularly  
98 surprising but we are not aware of any previous study which has directly demonstrated this. More  
99 surprising is the scale of the difference and the abruptness of the change demonstrated by our data,  
100 which has the non-linear characteristics of an ecological threshold (Groffman et al., 2006). An open  
101 question is whether this response reflects alternative stable states within the testate amoeba  
102 community *itself* or whether this is driven by similarly abrupt changes in plant communities and  
103 environmental conditions. Changes along the transects may be partly driven by moisture availability,  
104 paralleling evidence from many previous studies, but variance partitioning suggests that other  
105 factors must also be involved. The most distinct change along the transects is a loss of mixotrophic  
106 testate amoebae with tree cover, suggesting that reduced light penetration is an important factor  
107 driving the change in community (*cf.* Marcisz et al., 2014). Recent research has suggested that  
108 mixotrophic testate amoebae may make a non-trivial contribution to peatland primary production  
109 (Jassey et al., 2015). Although microbially-fixed carbon is likely to be a relatively small input  
110 compared to plant production, greater lability may make this pool disproportionately important in  
111 driving change in the microbial food-web. Our data also show that testate amoebae in open  
112 peatland tend to have larger apertures than those of forested peatland. This may reflect differences  
113 in available food sources with larger food items such as microalgae and ciliates likely to be more  
114 abundant in the open peatland. It is possible that tree encroachment might lead to greater grazing  
115 pressure from testate amoebae on bacteria due to the loss of alternative food types, with  
116 consequences for the biogeochemical processes those bacteria control.

117 Our study does not allow us to assess how quickly testate amoeba communities respond to tree  
118 establishment or whether these changes are reversible, but opens the way to palaeoecological

119 studies, which could address this question. Our dataset also provides a baseline for reassessments of  
120 these sites in the future.

121 On the basis of our results it seems probable that tree encroachment may lead to large changes in  
122 testate amoeba community with important implications for ecosystem processes such as microbial  
123 primary production and predation.

#### 124 ACKNOWLEDGEMENTS

125 Work in European Russia and data analysis was supported by the Russian Scientific Fund, grant 14-  
126 14-00891 to YuM. Fieldwork in Siberia was funded by the European Union Seventh Framework  
127 Programme under grant agreement no. 262693 [INTERACT] as part of the PEATEDGE (primary data  
128 collection), CliMireSiber (comparison dataset) and DAVOCCAP projects (participation of RA). UK-  
129 Russia research cooperation was supported by a Royal Society International Exchange grant  
130 (IE150173). During the research JR was supported by the Carnegie Trust for the Universities of  
131 Scotland through grant LG13STIR007 and AC was supported by a Natural Talent Apprenticeship from  
132 The Conservation Volunteers, funded by the Heritage Lottery Fund. RJP and RA are funded by the  
133 Leverhulme Trust through project RPG-2015-162. The comparison dataset was partially funded by  
134 grant PSPB-013/2010 (CLIMPEAT) through the Swiss Contribution to the enlarged European Union.

135 Author contributions: RJP, JR and YuM conceived the study. RJP, AC, JR, RA, EM, ANT, YuM, ML and  
136 EDL conducted fieldwork. AC, EM, KM and MZ analysed testate amoebae. RJP, YuM, ML, JGR and RA  
137 obtained funding and/or supervised research students. RJP conducted the data analysis and wrote  
138 the first draft of the paper. All authors commented on the draft and provided interpretation.

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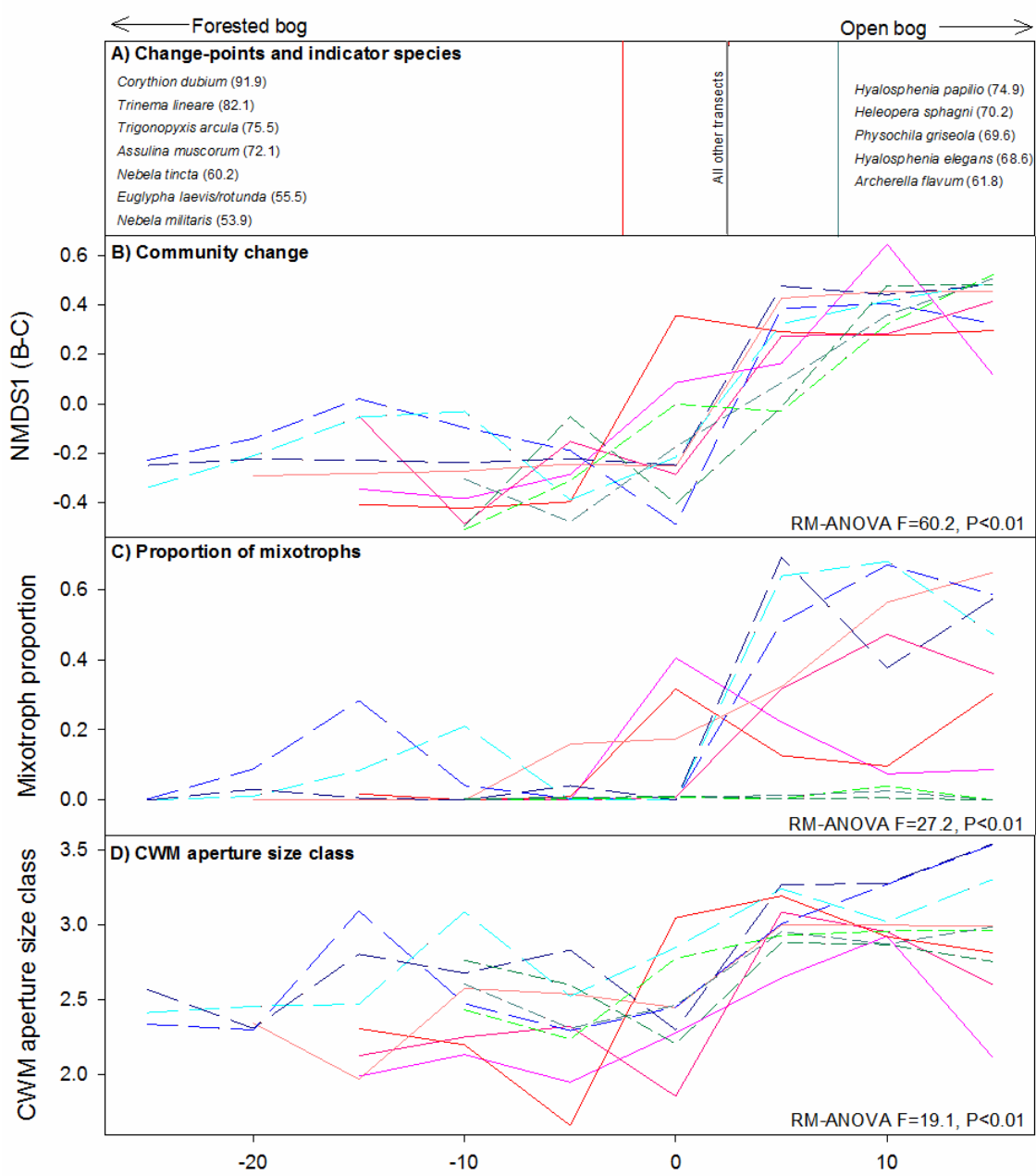
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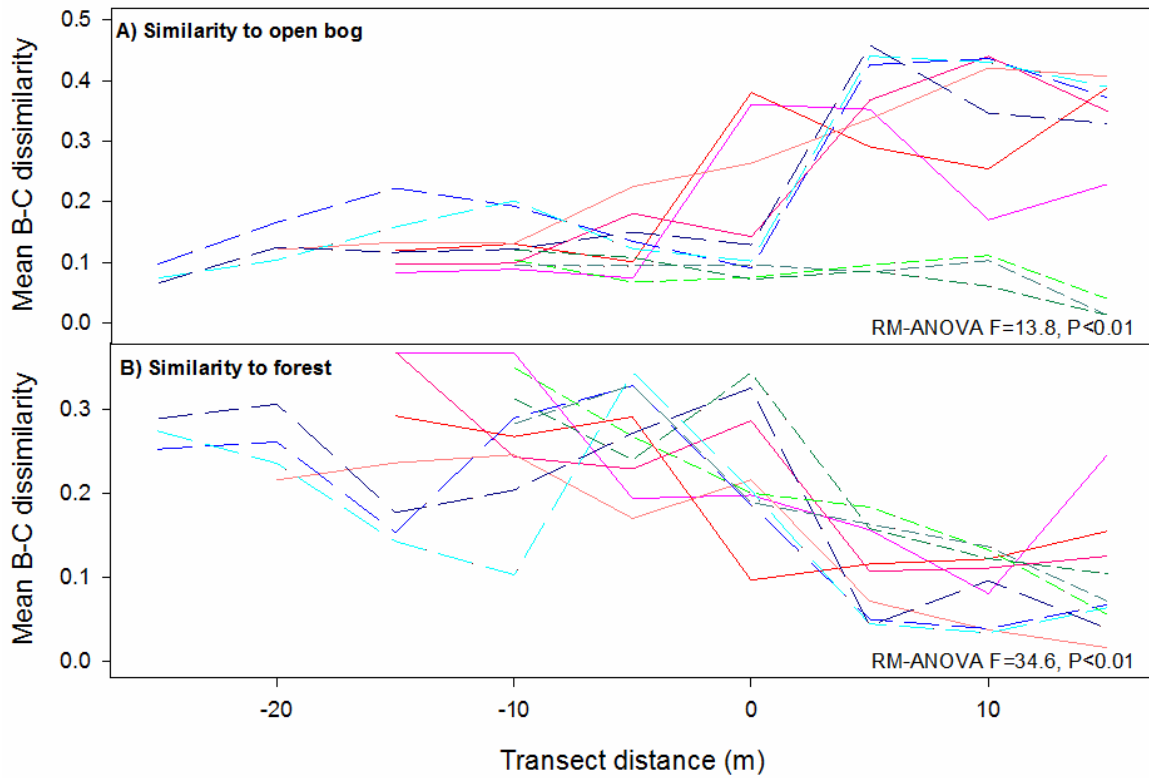
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220 Figure 1. Testate amoeba community change along forested to open bog transects. A) nCPA change-  
 221 points (vertical lines) and significant indicator species as identified by IndVal showing taxa IV>50 and  
 222 P<0.05. B) Community change as summarised by first axis scores from an NMDS ordination using  
 223 Bray-Curtis dissimilarity. C) Proportion of mixotrophic taxa. D) Community weighted mean (CWM)  
 224 aperture diameter in five size classes (1=0-10µm; 2=10-20µm; 3=20-30µm; 4=30-40µm; 5=>40µm).  
 225 In all plots lines represent individual transects from Mukhrino (shades of red); Morsovo (shades of  
 226 green) and Black River (shades of blue). In the bottom right of each plot are results of one way  
 227 repeated measures analysis of variance (RM-ANOVA) contrasting data either side of the nCPA  
 228 change-point.



230 Supplementary Table 1. Species abundance across the three study sites.

231 Supplementary Figure 1. A) Pairwise mean Bray-Curtis dissimilarity relative to testate amoebae  
232 communities of open bog (Lamentowicz et al. 2015b). B) Pairwise mean Bray-Curtis dissimilarity  
233 relative to testate amoeba communities of conifer forests on mineral soil (Malysheva, 2011). Details  
234 as for Figure 1.



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