Primer and database choice affect fungal functional but not biological diversity findings in a national soil survey

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Frontiers in Environmental Science

DOI:
10.3389/fenvs.2019.00173

Published: 01/11/2019

Peer reviewed version

Supplementary Material

Primer biases revealed in fungal functional but not biological diversity within a national soil survey

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S1. Creation of Aggregate Vegetation Classes

The land use classification used in this study was originally developed for the UK Countryside Survey in 1990 (Bunce et al., 1999). Briefly, vegetation data was collected from 508 individual randomly selected 1 km squares across the UK. Within each square, vegetation was recoreded in a number of plots placed either placed randomly or targeted to cover semi-natural habitats and along various landscape features such as field boundaries, hedges, and roads. This vegetation data was grouped into 100 vegetation

classes using the TWINSPAN programme (Hill, 1979b). Then, detrended correspondence analysis using DECORANA (Hill, 1979a) clustered these 100 vegetation classes into 8 Aggregate Vegetation Classes (AVCs), of which 7 were identified in the current study (Supplementary Table 1). The AVCs are ordered according to soil nutrient content (Bunce et al., 1999), from the high-nutrient crops to the low-nutrient bogs (see the order in Supplementary Table 1). Such a decline in soil nutrient content also implies both productivity and management intensity gradients.

~~**S2. Glastir Monitoring and Evaluation Programme**~~

~~The Glastir Monitoring and Evaluation Programme (GMEP) was designed to assess the outcomes of implementing the Welsh Government's Glastir agri-environment scheme. GMEP is a collaboration funded by the Welsh Government and the European Union. The GMEP programme is run by the NERC Centre for Ecology and Hydrology and is a collaboration between specialists from public research centres, universities, voluntary bodies, and consultancies. When active, GMEP was the largest and most in-depth monitoring programme measuring environmental state and change within the European Union (Emmett and the GMEP Team, 2017). GMEP follows a holistic ecosystem approach with a rolling annual survey conducted across areas both participating in and abstaining from Glastir. The results of the field survey were combined with national data and models to produce findings that inform stakeholders. The final report on GMEP data has been published and is accessible to the public (Emmett and the GMEP Team, 2017).~~

S2~~3~~. Soil maps in Wales

The soils at each sampling point were assigned to soil type using the National Soil Map and Soil Classification system (Cranfield University, 2004). This map and classification scheme is derived from Avery (1980) with revisions from Clayden and Hollis (1984). Soils were assigned to groups based on published soil maps and reconnaissance mapping of previously unsurveyed sites (for more detail see Cranfield University, 2004). Generally, soils in Wales are known to map poorly due to the high level of local spatial heterogeneity. We analysed soils at the major soil group level. There were 6 soil types that appeared across the GMEP sampling, which have been listed in increasing order of approximate moisture content throughout this study. They are: lithomorphic (ITS1 n = 13; 18S n = 13), brown (ITS1 n = 155; 18S n = 155), podzolic (ITS1 n = 109; 18S n = 113), surface-water gleys (ITS1 n = 80; 18S n = 82), ground-water gleys (ITS1 n = 12; 18S n = 11), and peats (ITS1 n = 44; 18S n = 48). In addition, each soil sample was categorised into an organic matter class based on loss-on-ignition (LOI) following the protocols of the 2007 Countryside Survey (Emmett et al., 2010) into four categories: mineral (0-8% LOI), humus-mineral (8-30% LOI), organo-mineral (30-60% LOI), and organic (60-100% LOI). The categories are listed in order of increasing organic matter content as follows: mineral (ITS1 n = 103; 18S n = 104), humus-mineral (ITS1 n = 228; 18S n = 232), organo-mineral (ITS1 n = 22; 18S n = 26), and organic (ITS1 n = 59; 18S n = 59).

S34. Pairwise differences of fungal OTU richness between land uses, soil organic matter, and soil type

As previously demonstrated in George et al. (*in press*), fungal OTU richness from ITS1 metabarcoding significantly ($F_{6, 258} = 39.87$, $p < 0.001$; Fig. 3A) declined from high to low productivity/management intensity. Fungal richness in Fertile grasslands was significantly greater than all other AVCs ($p < 0.001$) except Crops/weeds. Richness in Crops/weeds and Infertile grassland were significantly greater than all other land uses (all $p < 0.01$, except for Lowland wood $p = 0.002$ and $p = 0.01$, respectively). Fungal richness in Heath/bog was also significantly lower than that of Moorland grass-mosaics ($p < 0.001$) and both Lowland ($p = 0.02$) and Upland ($p = 0.007$) woodland AVCs.

In the 18S dataset, richness was also significantly ($F_{6,267} = 82.73$, $p < 0.001$) higher in more productive/managed land uses and declined along this gradient. Significantly greater fungal richness was observed in grassland AVCs than in all other AVCs ($p <0.001$ for all but Infertile grassland-Lowland wood $p = 0.03$) except Crops/weeds in the 18S dataset. Richness in Crops/weeds was also greater than Upland wood ($p = 0.02$). Heath/bog had the lowest richness, which was significantly lower than all other AVCs ($p < 0.001$). In addition, richness in both Crops/weeds, Lowland wood, and Upland wood was significantly greater than Moorland grass-mosaic ($p < 0.001$, $p = 0.002$, and $p = 0.04$, respectively).

For soil organic matter content, richness from both datasets richness was significantly greater ($F_{3, 259} = 48.13$, $p < 0.001$; $F_{3, 269} = 46.71$, $p < 0.001$; for ITS1 and 18S, respectively) in mineral and humus-mineral than all other classifications (Fig. 4). Specifically, fungal richness in mineral and humus-mineral soils was greater than that of organic (all $p < 0.001$) as well as organo-mineral soils ($p = 0.03$ and $p < 0.001$, for ITS1

and 18S, respectively). In both datasets, richness in organic soils was lower than that of organo-mineral soils ($p < 0.001$).

In the ITS1 dataset, fungal richness was significantly ($F_{5, 258} = 10.8$, $p < 0.001$) lower in peats than brown, podzolic, and both surface-water (all $p < 0.001$) and ground-water gley ($p = 0.002$) soils when soil type was assessed (Fig. S3A). However, aside from greater richness in brown soils than podzolic ($p = 0.009$) and surface-water gley ($p = 0.04$) soils, other differences were not apparent. A similar trend ($F_{5, 268} = 14.4$, $p < 0.001$) was observed in the 18S dataset (Fig. S3B). Here, fungal richness was again lower in Peats compared to brown, podzolic, ground- and surface-water gley soils ($p < 0.001$). Brown soils had the highest fungal richness, which was also greater than that of podzolic ($p < 0.001$) and lithomorphic ($p = 0.02$) soils.

Across land uses, significant differences were observed in the richness of saprotrophic fungi in both the ITS1 ($F_{6,258} = 25.14$, $p < 0.001$) and 18S ($F_{6, 267} = 31.10$, $p < 0.001$) data; however, there were differences between datasets (Fig. 8). In the ITS1 dataset, richness followed the same trend as overall fungal richness, with the highest and lowest values in the Crops/weeds and Heath/bog AVCs respectively (Fig. 8A). Saprotroph richness was significantly greater in Crops/weeds than Infertile grassland ($p = 0.001$), Lowland wood, Upland wood, Moorland grass-mosaic, and Heath/bog (all $p < 0.001$). Similarly, saprotroph richness was significantly greater than all groups except Lowland wood (all $p < 0.001$). Richness of saprotophs in Moorland grass-mosaic and Heath/bog sites was also significantly lower than that of Infertile grassland areas (both $p < 0.001$). Although this pattern was preserved in the 18S data, richness of saprotrophs was much more even in this case. Indeed, rather than the linear decline of richness along

the productivity gradient, there appeared to be 3 distinct levels in the data associated with grassland/agricultural sites, woodlands, and bogs. Saprotroph richness was significantly lower in Heath/bog sites than all other AVCs (all $p < 0.001$) and highest in grasslands. There were significant differences between saprotroph richness in grasslands and Moorland grass-mosaic and wood AVCs (all $p < 0.001$ except Fertile grassland – Lowland wood $p = 0.046$ and Infertile grassland – Lowland wood $p = 0.001$).

In the ITS1 dataset, each organic matter class was significantly ($F_{3, 260} = 32.86$, $p < 0.001$) different from the others (Fig. 9). Mineral soils had the highest saptrotroph richness when compared to all others ($p < 0.001$ except for humus-mineral $p = 0.003$). Saprotroph richness in humus-mineral soils was greater than both organo-mineral ($p = 0.03$) and organic soils ($p < 0.001$) and richness in organo-mineral soils greater than organic ($p = 0.03$) soils (Fig. 9A). In the 18S data, saprotroph richness was significantly ($F_{3, 269} = 41.13$, $p < 0.001$) higher in mineral and humus-mineral soils than organo-mineral and organic (all $p < 0.001$ except mineral – organo-mineral $p = 0.02$) soils (Fig. 9B). Again, the overarching trend of fungal richness was not apparent when samples were grouped by soil type. Although there were significant differences across soil types in both the ITS1 ($F_{5, 259} = 9.7$, $p < 0.001$) and 18S ($F_{5, 268} = 10.73$, $p < 0.001$) datasets, these differences did demonstrate consistent patterns across soil types (Fig. S10). Richness of saprotrophs mirrored the exact trend as total richness in the ITS1 dataset. Saprotroph richness was significantly lower in peats than brown ($p < 0.001$), podzolic ($p = 0.045$), and both surface-water and ground-water gley (both $p = 0.01$) soils (Fig. S10A). Saprotroph richness in was higher in brown soils than Podzolic ($p = 0.009$) and surface-water gley ($p = 0.03$) soils, other differences were not apparent. In the 18S dataset,

saprotroph richness was lower than in all other soils except lithomorphic (all $p < 0.001$; Fig. S10B).

In the ITS1 data, significantly ($F_{6, 258} = 26.11$, $p < 0.001$) greater pathotroph richness values were observed in Crops/weeds and grassland samples in comparison to the other AVC categories (Fig. 8A). Richness of pathotrophs was significantly greater in Crops/weeds sites than all other AVCs (all $p < 0.001$, except Fertile grassland $p = 0.02$). Similarly, richness was greater in the Fertile grasslands than all other remaining land uses (all $p < 0.001$, except Infertile grassland $p = 0.002$). Richness of pathrotrophs in Infertile grasslands was also significantly greater than in all remaining AVCs except Heath/bog ($p < 0.001$). Again, this trend was present, though not as stark, in the 18S dataset (Fig. 8B). Significant differences ($F_{6, 267} = 52.26$, $p < 0.001$) were observed between AVCs, with the highest richness of pathotrophs occurring in the Fertile grassland and Crop/weeds land uses. Pathotroph richness was greater in Fertile grasslands than all other AVCs (all $p < 0.001$, except Lowland wood $p = 0.001$) but Crops/weeds. Richness in Crops/weeds, Infertile grassland, and Lowland wood samples was greater than Moorland grass-mosaic and (all $p < 0.001$, except Lowland wood – Moorland grass-mosaic $p = 0.002$), Upland wood (all $p < 0.001$, except Lowland wood – Upland wood $p = 0.04$), and Heath/bog (all $p < 0.001$). Additionally, pathotroph richness in Heath/bog sites was lower than Moorland grass-mosaic samples ($p = 0.01$).

When grouped by organic matter class, significant differences were also observed in pathotroph richness in the ITS1 ($F_{3, 250} = 24.91$, $p < 0.001$) and 18S ($_{F3, 269} = 30.49$, $p < 0.001$) datasets. However, in this case the trends were more apparent in the 18S data than the ITS1 data (Fig. 9). Pathotroph richness was significantly greater in mineral than

humus-mineral ($p = 0.03$) soils and was significantly lower in organic soils when compared to all others (all $p < 0.001$) in the ITS1 data (Fig. 9A). However, all organic matter classifications were statistically different from each other in the 18S data (Fig. 8B), in descending order from mineral to peat soils (all $p < 0.001$, except organo-mineral – organic $p = 0.03$, humus-mineral – mineral and organo-mineral both $p = 0.001$). Again, trends were less clear across soil types (Fig. S#). Peat soils had significantly ($F_{5, 259} = 6.93$, $p < 0.001$) lower pathotroph richness than brown ($p < 0.001$), podzolic, ground-water gley (both $p = 0.002$), and surface-water gley ($p = 0.007$) soils (Fig. S10A) in the ITS1 data. Differences between pathotrophic fungi across soil types were more similar to those observed in other groups in the 18S data (Fig. S10B). Pathotroph richness was significantly ($F_{5, 268} = 13.6$, $p < 0.001$) greater in brown soils than lithomorphic ($p = 0.03$), podzolic ($p = 0.02$), and peat ($p < 0.001$) soils. Richness in peats was again significantly lower than that of podzolic, surface- and ground-water gley (all $p < 0.001$) soils.

Although significant differences were apparent in both the ITS1 ($F_{6, 258} = 14.88$, $p < 0.001$) and 18S ($F_{6, 267} = 55.13$, $p < 0.001$) datasets they were by no means identical (Fig. 7). Symbiotroph richness was higher in Lowland wood sites in the ITS1 than all other AVCs (all $p < 0.001$ except Upland wood $p = 0.046$. Symbiotroph richness was also higher in Upland wood sites than the Infertile grassland, Moorland grass-mosaic, and Heath/bog AVCs (all $p < 0.001$; Fig. 8A). This trend was not apparent in the 18S dataset however (Fig. 8B). Here richness of symbiotrophs was significantly greater in grasslands AVCs than all other AVCs (all $p < 0.001$). Similarly, richness of symbiotrophs from

Heath/bog sites was significantly lower than those of Lowland wood ($p = 0.02$), Upland wood, Crops/weeds, and Moorland grass-mosaic (all $p < 0.001$).

Across organic matter classes, the previously described trend of decreasing richness with increasing organic matter content held true in the 18S data ($F_{3, 269} = 36.28$, $p < 0.001$; Fig. 8B), with no significant differences observed in the ITS1 dataset ($F_{3, 260} = 1.88$, $p = 0.13$; Fig 9A). In the 18S data, richness of symbiotrophs was significantly greater in mineral and humus-mineral soils when compared to organo-mineral ($p = 0.002$, $p = 0.04$, respectively) and organic ($p < 0.001$) soils (Fig. 9B). There were also no significant differences ($F_{5, 259} = 1.43$, $p = 0.21$) in symbiotroph richness across soil types in ITS1 data (Fig. S#A), though there were in 18S data ($F_{5, 259} = 12.52$, $p < 0.001$; Fig. S#B). As described previously, in this case richness was lower in peat soils than in ground-water gley ($p = 0.02$), surface-water gley, podzolic, and brown (all $p < 0.001$) soils. Additionally, symbiotroph richness was higher in brown soils than in podzolic ($p = 0.02$) and lithomorphic ($p = 0.046$ soils.

There were significant ($F_{6, 244} = 33.47$, $p < 0.001$) differences in richness of Glomeromycota across land uses, though they appeared, like the saprotroph richness to be tiered between grasslands, woods, and bogs (Fig. 10A). Richness of Glomeromycetes was higher in Fertile and Infertile grasslands than all other AVCs (all $p < 0.001$, except Moorland grass-mosaic $p = 0.04$ and $p = 0.008$, respectively) except Crops/weeds. Richness in Heath/bog sites was significantly lower than Moorland grass-mosaic, Lowland wood (both $p < 0.001$), and Upland wood ($p = 0.01$). In addition, significant differences were observed between Upland wood and Moorland-grass ($p < 0.001$).

Again, when grouped by organic matter class (Fig. 10B) and soil type (Fig. 10C) Glomeromycetes richness followed the same trend saprotrophs and symbiotrophs from the 18S dataset. Richness was significantly ($F_{3, 246} = 37.65$, $p < 0.001$) greater in mineral and humus-mineral soils than all other organic matter classes ($p < 0.001$). Richness was also lower in organic soils than organo-mineral soils ($p = 0.002$). Across soil types, richness of Glomeromycetes was significantly ($F_{5, 245} = 8.65$, $p < 0.001$) lower in peat soils when compared to brown, podzolic, surface-water (all $p < 0.001$) and ground-water gley ($p = 0.004$) soils.

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in an ordered two-way table by classification of the individuals and attributes. (Cornell University, Ithaca).

Supplementary Tables

Supplementary Table 1. Description of Aggregate Vegetation Classes identified in this study. Adapted from Smart et al. (2003).

Aggregate Vegetation Class	Description
Crops/weeds (ITS1 n = 9; 18S n = 8)	Communities on disturbed or cultivated land, including weedy, horticultural, and species-poor arable land.
Fertile grassland (ITS1 n = 97; 18S n = 96)	Improved or semi-improved grassland. Usually with high nutrient inputs and cut more than once a year.
Infertile grassland (ITS1 n = 156; 18S n = 158)	Semi-improved to unimproved, less productive grasslands, species-rich grasslands including wet or dry and acidic to basic variations.
Lowland wood (ITS1 n = 17, 18S n = 16)	Dominated by trees and shrubs in neutral or basic lowlands, scrublands, and hedgerows.
Upland wood (ITS1 n = 43; 18S n = 43)	Commonly acidic conifer plantations, scrubland and semi-natural broadleaved woods in the uplands.
Moorland grass/mosaic (ITS1 n = 44; 18S n = 53)	Grass-dominated upland pasture, commonly with a long history of livestock grazing.
Heath/bog (ITS1 n = 47; 18S n = 48)	Heather dominated, commonly upland landscapes, including dry heath and bogs.

Supplementary Table 2. Mean values (± SE) of soil physical and chemical variables of each organic matter class. Following normalisation on selected variables (see below) ANOVAs and Tukey's *post-hoc* tests were performed. Results are as follows: total C ($F_{3\ 431}$ = 613.22 p < 0.001), total N ($F_{3\ 431}$ = 564.38, p < 0.001), C :N ratio ($F_{3,\ 431}$ = 175.81, p < 0.001), total P ($F_{3,\ 428}$ = 8.46, p < 0.001), organic matter ($F_{3,\ 432}$ = 1358.5, p < 0.001), pH ($F_{3,\ 432}$ = 83.53, p < 0.001), soil water repellency ($F_{3\ 432}$ = 41.39, p < 0.001), volumetric water content ($F_{3,\ 431}$ = 61.93, p < 0.001), soil bound water ($F_{3\ 432}$ = 626.58, p < 0.001), rock volume ($F_{3,\ 431}$ = 19.55, p < 0.001), bulk density ($F_{3,\ 431}$ = 485.08, p < 0.001), clay content ($F_{3,\ 347}$ = 44.86, p < 0.001), sand content ($F_{3,\ 347}$ = 21.56, p < 0.001), elevation ($F_{3,\ 432}$ = 100.34, p < 0.001), mean annual precipitation ($F_{3,\ 432}$ = 69.38, p < 0.001), and temperature ($F_{3\ 432}$ = 0.69, p = 0.56).

Environmental variable	Mineral	Humus-mineral	Organo-mineral	Organic
Total C (%)^{L}	3.14 (± 0.10)d	6.80 (± 0.20)c	39.38 (± 1.23)a	25.27 (± 0.76)b
Total N (%)^{L}	0.29 (± 0.01)d	0.55 (± 0.01)c	1.39 (± 0.06)b	1.83 (± 0.06)a
C:N ratio^{S}	11.14 (± 0.25)d	12.22 (± 0.22)c	18.5 (± 0.89)b	22.1717 (± 0.88)a
Total P (mg/kg)^{S}	855.67 (± 36.98)b	1085.32 (± 34.16)a	1208.15 (± 96.40)a	915.82 (± 47.6)a
Organic matter (% LOI)^{L}	6.18 (± 0.13)d	13.06 (± 0.31)c	44.2 (± 1.58)b	75.64 (± 1.31)a
pH ($CaCl_2$)	5.04 (± 0.08)a	4.55 (± 0.05)b	3.46 (± 0.16)c	3.46 (± 0.06)d
Soil water repellency*	208.08 (± 42.02)c	1589.51 (± 292.96)b	3757.2 (± 688.25)a	3939.5 (± 759.14)a
Volumetric water content (m^3/m^3)	0.29 (± 0.01)c	0.36 (± 0.01)b	0.47 (± 0.04)a	0.65 (± 0.02)a
Soil bound water (g water per g of dry soil)^{L}	2.0 (± 0.06)d	3.26 (± 0.07)c	7.3 (± 0.47)b	9.18 (± 0.28)a
Rock volume (mL)	4.12 (± 0.39)b	5.82 (± 0.36)a	2.84 (± 0.69)bc	1.11 (± 0.2)c
Bulk density (g/cm^3)	1.02 (± 0.01)a	0.71 (± 0.01)b	0.24 (± 0.02)c	0.13 (± 0.01)d
Clay content (%)^{A}	20.43 (± 0.67)a	24.2 (± 0.52)a	10.49 (± 0.88)b	8.14 (± 4.94)c
Sand content (%)^{A}	36.68 (± 1.94)b	25.56 (± 0.97)c	45.24 (± 3.81)b	66.08 (± 12.4)a
Elevation (m)	106.04 (± 8.5)d	201.39 (± 9.18)c	355.3 2 (± 34.17)a	325.18 (± 13.45)b
Mean annual precipitation (mL)	1072.06 (± 29.86)c	1279.39 (± 27.91)b	1884.47 (± 93.43)a	1806.5 (± 39.78)a
Temperature (°C)	13.79 (± 0.41)a	13.14 (± 0.23)a	14.22 (± 0.57)a	12.07 (± 0.3)a

Note: ^{A} denotes Aitchison's log_{10}-ratio transformation; ^{L} denotes log_{10}-transformation; square-root-transformation; *Soil water repellency was derived from median water drop penetration times (s) and log_{10} transformed.

Supplementary Table 3. Mean values (± SE) of soil physical and chemical variables of each soil type. Following normalisation on selected variables (see below) ANOVAs and Tukey's *post-hoc* tests were performed. Results are as follows: total C ($F_{5, 429} = 52.5$ $p < 0.001$), total N ($F_{5, 429} = 43.8$, $p < 0.001$), C :N ratio ($F_{5, 429} = 38.12$, $p < 0.001$), total P ($F_{5, 426} = 1.89$, $p = 0.1$), organic matter ($F_{5, 430} = 61.01$, $p < 0.001$), pH ($F_{5, 430} = 34.51$, $p < 0.001$), soil water repellency ($F_{5, 430} = 10.16$, $p < 0.001$), volumetric water content ($F_{5, 429} = 23.07$, $p < 0.001$), soil bound water ($F_{5\ 430} = 56.94$, $p < 0.001$), rock volume ($F_{5, 429} = 7.31$, $p < 0.001$), bulk density ($F_{5, 429} = 48.6$, $p < 0.001$), clay content ($F_{5, 346} = 4.18$, $p = 0.01$), sand content ($F_{5, 346} = 3.42$, $p = 0.01$), elevation ($F_{5, 431} = 61.73$, $p < 0.001$), mean annual precipitation ($F_{5, 431} = 43.76$, $p < 0.001$), and temperature ($F_{5, 431} = 1.38$, $p = 0.23$).

Environmental variable	Lithomorphic	Brown	Podzolic	Surface-water gleys	Ground-water gleys	Peats
Total C (%)^{L}	12.55 (± 4.98)b	4.68 (± 0.33)d	9.0 (± 1.31)b	7.62 (± 1.12)c	8.26 (± 1.68)bcd	9.74 (± 2.46)a
Total N (%)^{L}	0.69 (± 0.26)d	0.41 (± 0.02)d	0.65 (± 0.05)d	0.55 (± 0.06)d	0.68 (± 0.08)c	0.61 (± 0.1)b
C:N ratio^{S}	15.66 (± 1.39)b	11.21 (± 0.24)c	12.98 (± 0.52)b	12.92 (± 0.52)b	11.56 (± 1.4)bc	15.5 (± 0.9)a
Total P (mg/kg)^{S}	1027.75 (± 191.25)a	1043.18 (± 37.88)a	1146.16 (± 50.2)a	879.98 (± 46.33)a	974.55 (± 76.97)a	699.94 (± 59.7)a
Organic matter (% LOI)^{L}	22.98 (± 8.73)b	9.27 (± 0.67)d	17.16 (± 0.56)b	13.76 (± 1.7)c	15.27 (± 4.22)bcd	18.7 (± 3.72)a
pH ($CaCl_2$)	4.33 (± 0.22)bcd	4.89 (± 0.07)a	4.21 (± 0.07)b	4.73 (± 0.1)ab	5.05 (± 0.29)a	3.96 (± 0.1)d
Soil water repellency^{*L}	1261.43 (± 1035.2)ac	651.35 (± 144.0)c	1623.78 (± 419.91)ab	1329.46 (± 332.21)bc	4203.08 (± 2721.78)ab	3941.17 (± 1025.53)a
Volumetric water content (m^3/m^3)	0.47 (± 0.05)ac	0.31 (± 0.01)d	0.37 (± 0.02)c	0.38 (± 0.02)bc	0.4 (± 0.04)bcd	0.43 (± 0.02)a
Soil bound water (g water per g of dry soil)	3.98 (± 0.89)b	2.66 (± 0.1)d	3.62 (± 0.29)bc	3.15 (± 0.25)cd	4.26 (± 0.54)bd	3.68 (± 0.51)a
Rock volume (mL)	4.05 (± 0.92)ab	5.52 (± 0.43)a	6.21 (± 0.47)a	3.49 (± 0.49)b	5.0 (± 1.34)ab	2.85 (± 0.32)b
Bulk density (g/cm^3)	3.98 (± 0.09)cd	2.66 (± 0.02)b	3.62 (± 0.03)c	3.15 (± 0.03)a	4.26 (± 0.08)bcd	3.68 (± 0.04)d
Clay content (%)^{A}	15.27 (± 5.33)bc	21.99 (± 0.57)b	22.76 (± 0.92)ac	22.9 (± 0.92)ab	26.28 (± 3.18)ac	19.29 (± 3.17)c
Sand content (%)^{A}	51.49 (± 12.5)a	32.01 (± 1.41)b	27.33 (± 1.8)b	29.09 (± 2.01)b	25.33 (± 2.96)b	27.89 (± 7.52)b
Elevation (m)	219.18 (± 39.49)bc	125.19 (± 7.91)d	268.59 (± 14.32)b	177.78 (± 15.52)c	33.57 (± 15.72)d	363.58 (± 17.46)a
Mean annual precipitation (mL)	1643.0 (± 144.42)ab	1093.36 (± 14.6)d	1525.41 (± 46.68)b	1223.14 (± 48.45)c	949.58 (± 34.2)b	1352.08 (± 66.26)a
Temperature (°C)	15.7 (± 0.63)a	13.24 (± 0.36)a	13.62 (± 0.29)a	13.05 (± 0.33)a	15.2 (± 0.94)a	11.71 (± 0.31)a

Note: ^{A} denotes Aitchison's $\log_{10}$-ratio transformation; ^{L} denotes $\log_{10}$-transformation; square-root-transformation; *Soil water repellency was derived from median water drop penetration times (s) and $\log_{10}$ transformed.

Supplementary Table 4. Richness of OTUs at the class-level that appear in both the ITS1 and 18S datasets.

Class	Number of OTUs in ITS1	Number of OTUs in 18S
Agaricomycetes	1858	646
Agaricostilbomycetes	1	36
Archaeorhizomycetes	6	129
Arthoniomycetes	1	5
Chytridiomycetes	2	1001
Cystobasidiomycetes	2	2
Dothideomycetes	326	91
Eurotiomycetes	472	86
Exobasidiomycetes	4	40
Geoglossomycetes	7	1
Glomeromycetes	2	162
Lecanoromycetes	22	29
Leotiomycetes	422	66
Microbotryomycetes	40	65
Monoblepharidomycetes	5	27
Orbiliomycetes	31	6
Pezizomycetes	79	49
Pucciniomycetes	2	29
Saccharomycetes	23	106
Sordariomycetes	915	417
Tremellomycetes	25	181
Ustilaginomycetes	3	7

Supplementary Table 5. Richness of OTUs at the class-level that appear in only the ITS1 dataset.

Class	Number of OTUs
Archaeosporomycetes	1
Calcarisporiellomycetes	6
Endogonomycetes	19
Geminibasidiomycetes	2
GS17	1
Kickxellomycetes	1
Malasseziomycetes	23
Mortierellomycetes	128
Mucoromycetes	55
Mucoromycotiunidentified_cls_Incertae_sedis	2
Paraglomeromycetes	3
Rhizophlyctidomycetes	5
Rhizophydiomycetes	11
Spizellomycetes	8
Umbelopsidomycetes	17
Unidentified	1125
Xylonomycetes	10
Zoopagomycetes	2

Supplementary Table 6. Richness of OTUs at the class-level that appear in only the 18S dataset.

Class	Number of OTUs
Ambiguous taxa*	208
Atractiellomycetes	8
Basidomycetes	3
Dacrymycetes	6
Incertae Sedis	715
Laboulbeniomycetes	17
Lichinomycetes	5
LKM11	182
Microsporidia	1
Neocallimastigomycetes	6
Pneumocystidomycetes	1
Schizosaccharomycetes	3
Taphrinomycetes	9

*This includes OTUs identified as “ambiguous taxa”, “Amb-18S-784”, and “Acomycota sp. MUT 4926”.

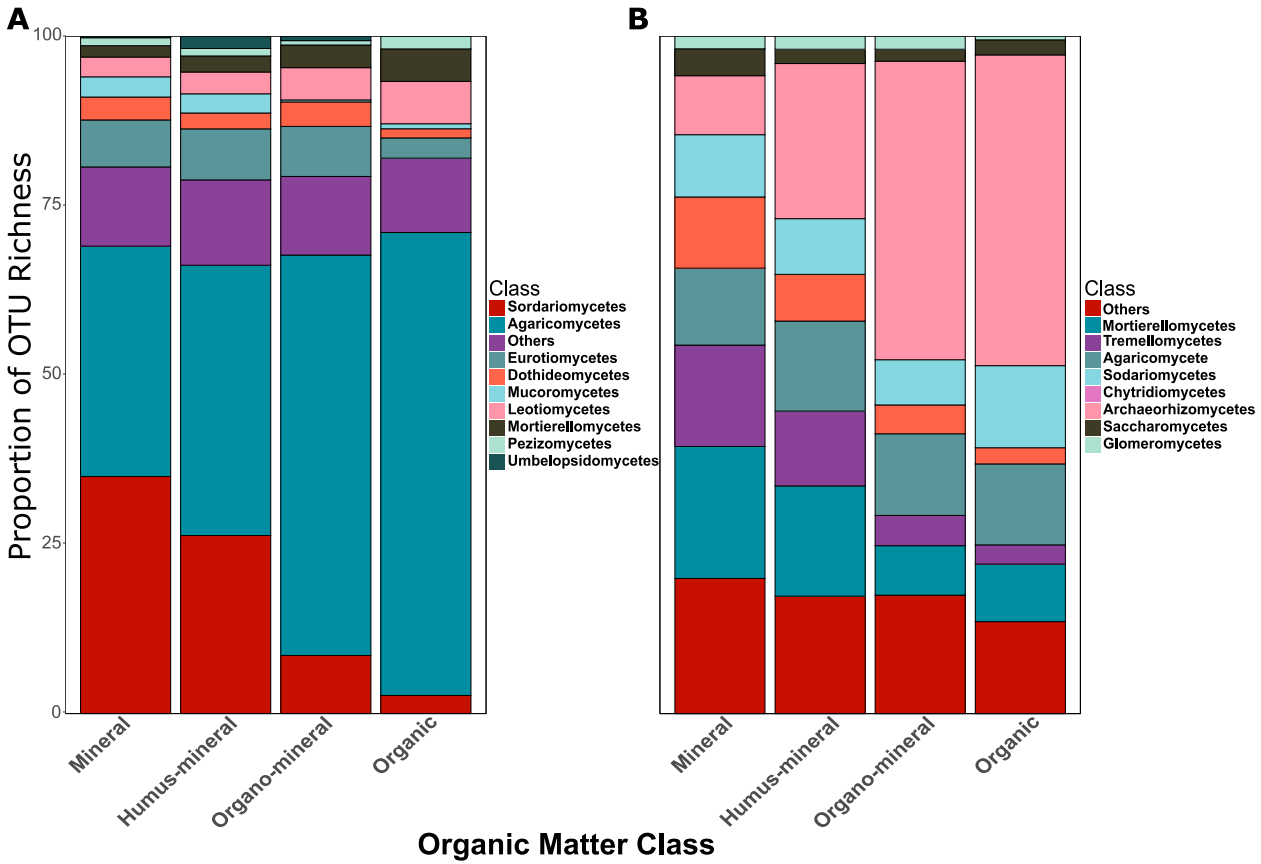

Fig. S1. Proportionate abundances of fungal OTUs for **A)** ITS1 and **B)** 18S data across the different organic matter classes. Organic matter classes are ordered by increasing percent organic matter.

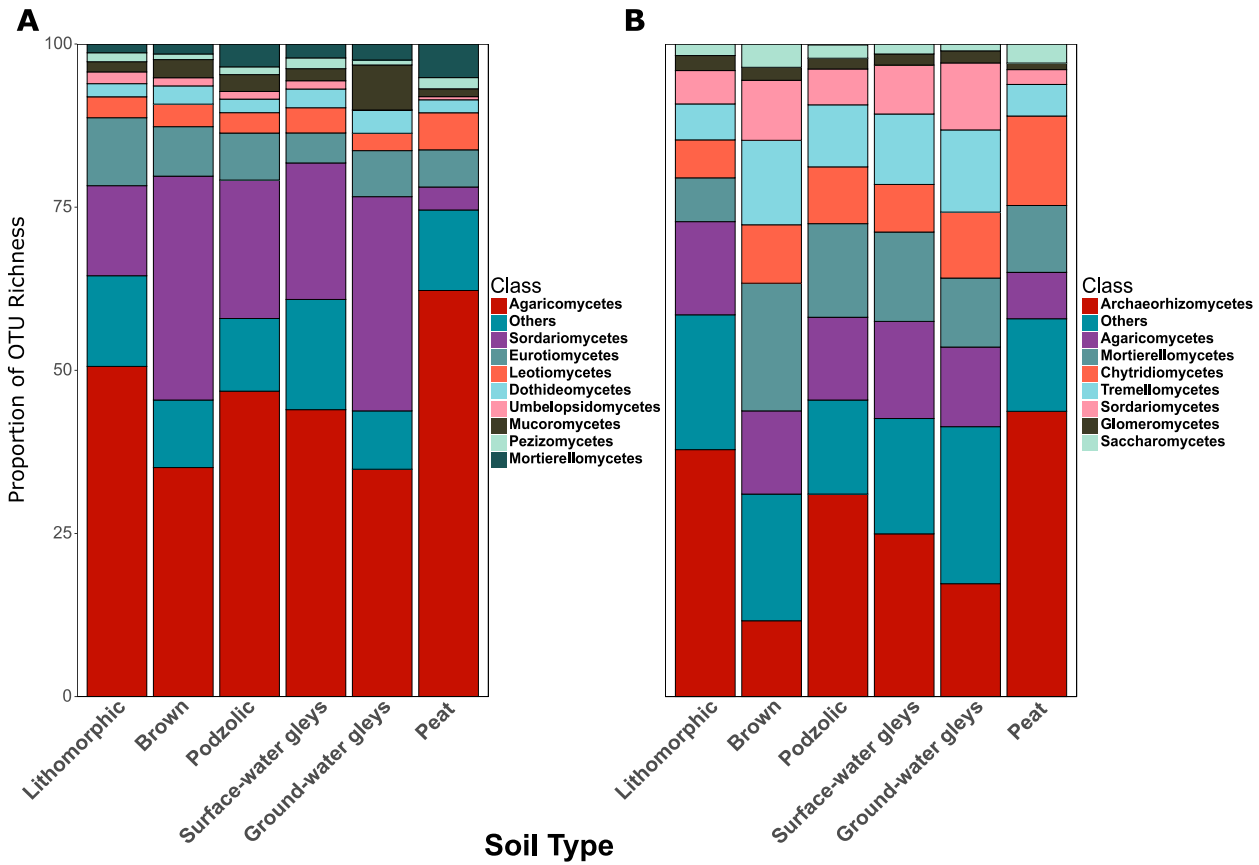

Fig. S2. Proportionate abundances of fungal OTUs for **A)** ITS1 and **B)** 18S data across the different soil types. Soil types are ordered by increasing approximate percent moisture content.

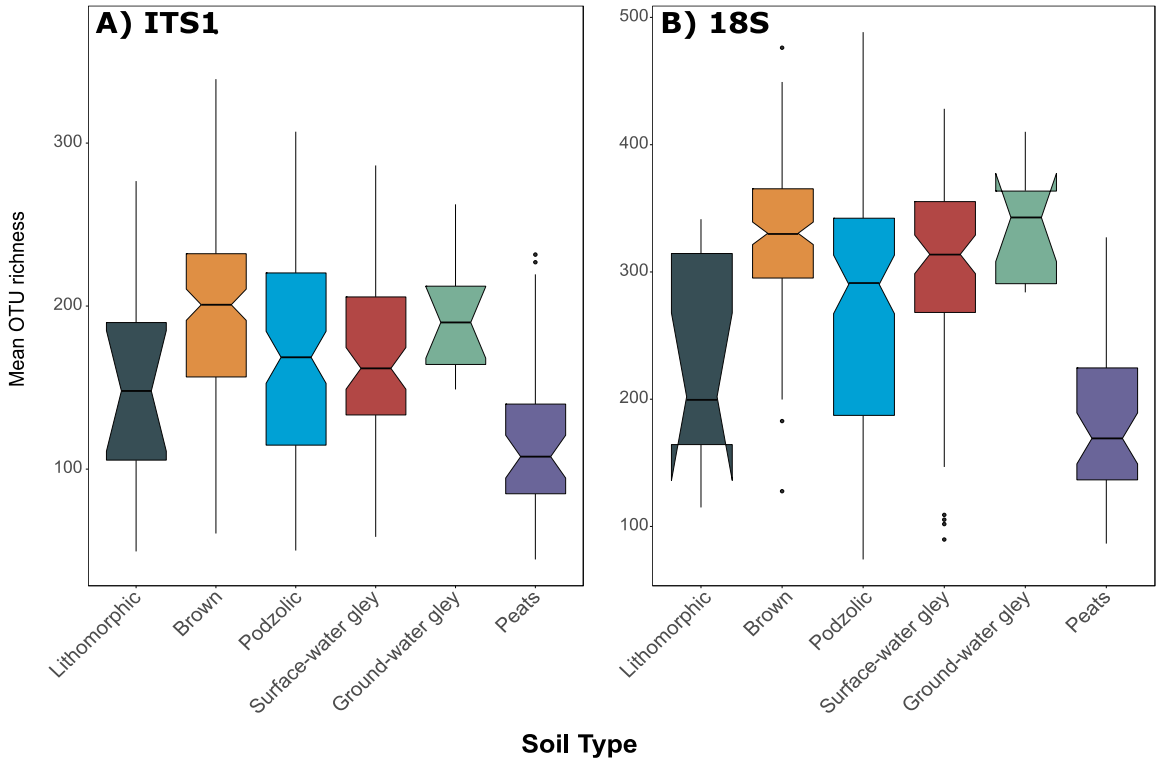

Fig. S3. Boxplots of fungal OTU richness for **A)** ITS1 and **B)** 18S datasets plotted against soil type. Soil types are ordered by increasing approximate moisture content. Boxes cover the first and third quartiles and horizontal lines denote the median. Black dots represent outliers beyond the whiskers, which cover 1.5X the interquartile range.

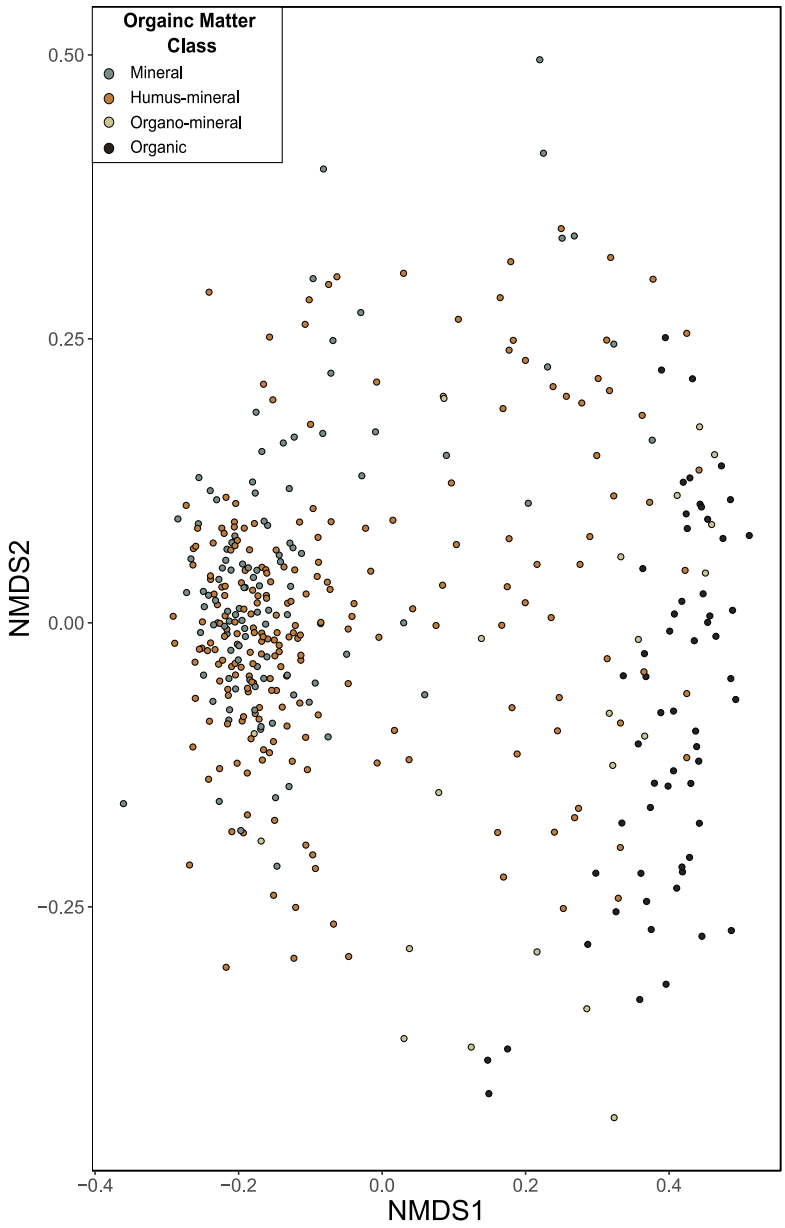

Fig. S4. Non-metric dimensional scaling ordinations of fungal community composition from ITS1 data across GMEP sites (stress = 0.13). Samples are coloured by organic matter class. Results of both PERMANOVA ($F_{3,408}$ = 9.34, p = 0.001) and of testing dispersion of variances ($F_{3,408}$ = 10.66, p = 0.001) were significant.

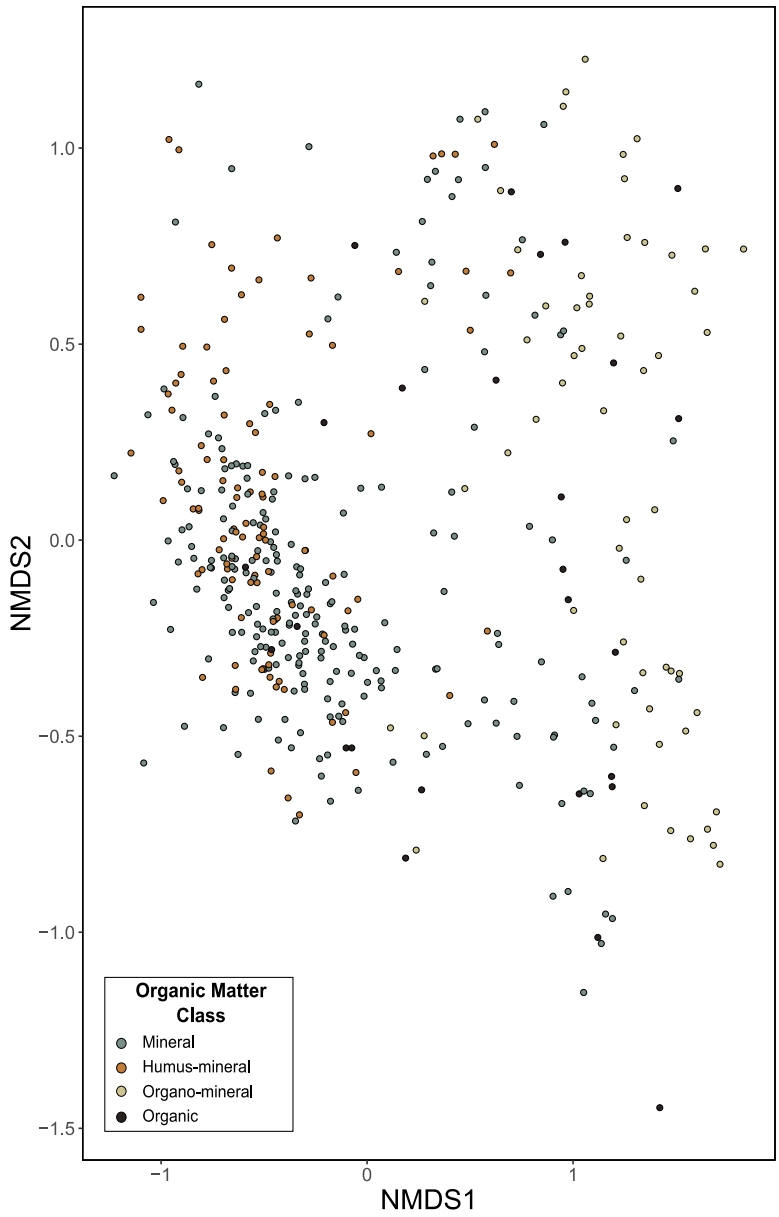

Fig. S5. Non-metric dimensional scaling ordinations of fungal community composition from 18S data across GMEP sites (stress = 0.11). Samples are coloured by organic matter class. Results of both PERMANOVA ($F_{3,417}$ = 13.06, p = 0.001) and of testing dispersion of variances ($F_{3,417}$ = 8.69, p = 0.001) were significant.

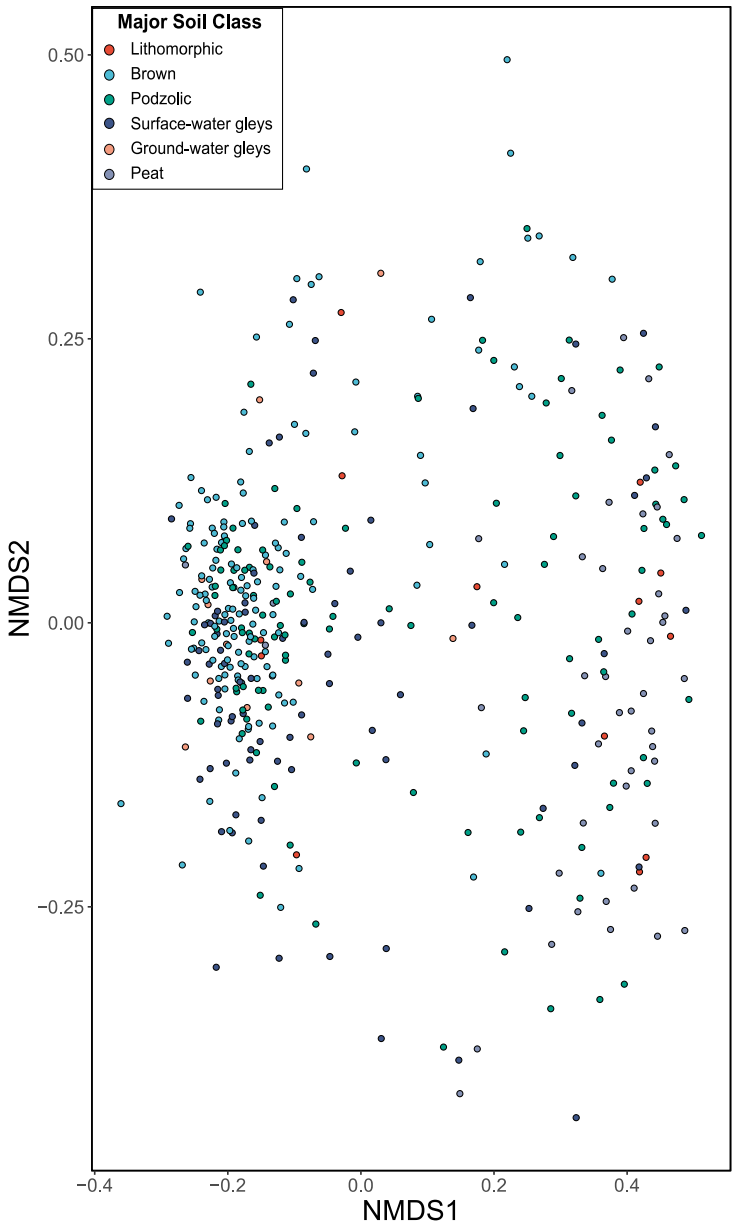

Fig. S6. Non-metric dimensional scaling ordinations of fungal community composition from ITS1 data across GMEP sites (stress = 0.13). Samples are coloured by soil type. Results of both PERMANOVA ($F_{5,407} = 4.44$, $p = 0.001$) and of testing dispersion of variances ($F_{5,407} = 9.72$, $p = 0.001$) were significant.

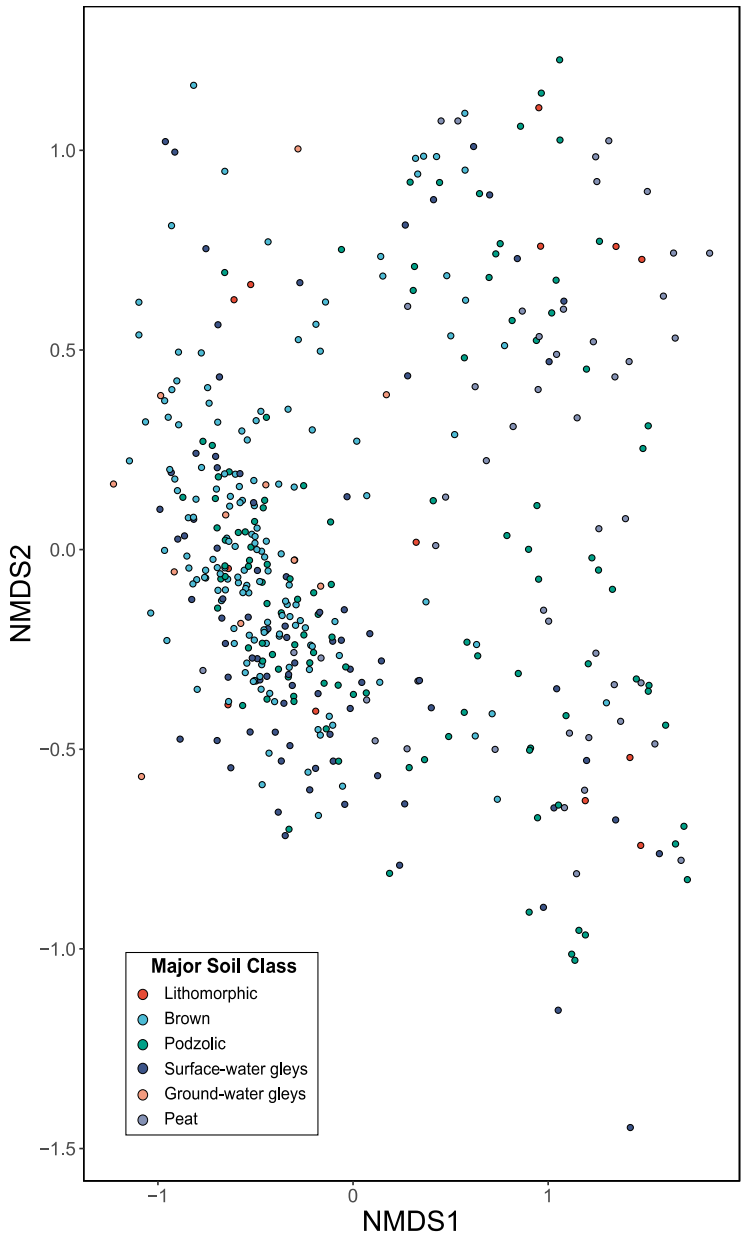

Fig. S7. Non-metric dimensional scaling ordinations of fungal community composition from 18S data across GMEP sites (stress = 0.11). Samples are coloured by soil type. Results of both PERMANOVA ($F_{5,416} = 6.0$, $p = 0.001$) and of testing dispersion of variances ($F_{5,416} = 6.91$, $p = 0.001$) were significant.

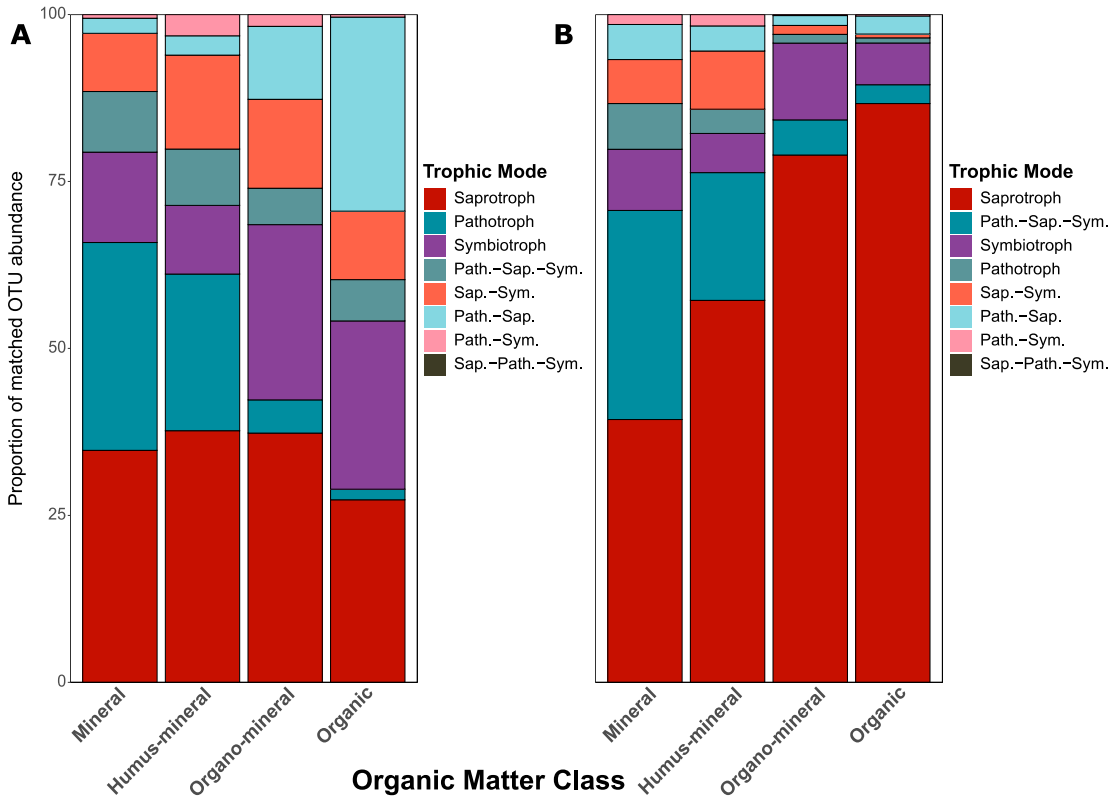

Fig. S8. Proportionate abundances of fungal OTUs matched to FUNGuild trophic groups for **A)** ITS1 and **B)** 18S data across organic matter classes. Organic matter classes are ordered by increasing percent organic matter. Abbreviations for multi-trophic mode groups are as follows: Path.-Sap. (Pathotroph-Saprotroph); Path.-Sap.-Sym. (Pathotroph-Saprotroph-Symbiotroph); Path.-Sym. (Pathotroph-Symbiotroph); Sap.-Path.-Sym (Saprotroph-Pathotroph-Symbiotroph); Sap.-Sym. (Saprotroph-Symbiotroph).

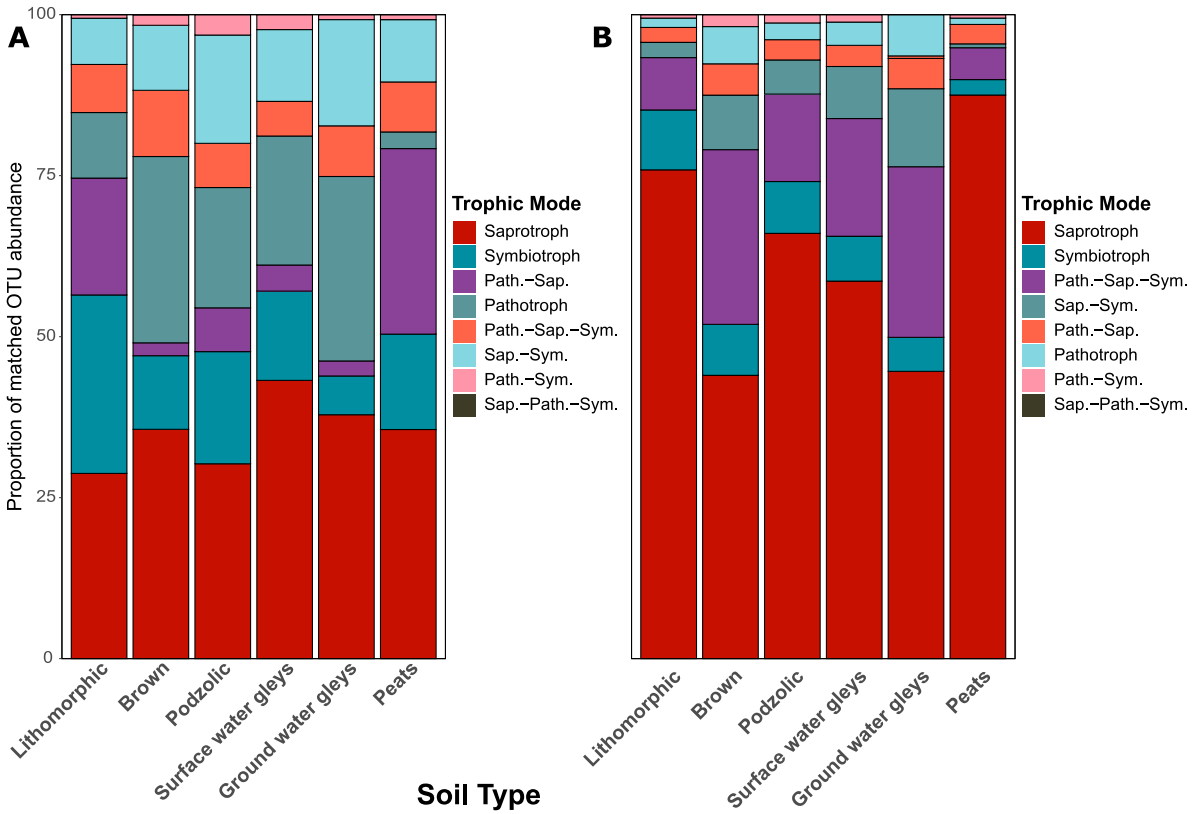

Fig. S9. Proportionate abundances of fungal OTUs matched to FUNGuild trophic groups for **A)** ITS1 and **B)** 18S data across soil types. Soil types are ordered by increasing approximate moisture content. Abbreviations for multi-trophic mode groups are as follows: Path.-Sap. (Pathotroph-Saprotroph); Path.-Sap.-Sym. (Pathotroph-Saprotroph-Symbiotroph); Path.-Sym. (Pathotroph-Symbiotroph); Sap.-Path.-Sym (Saprotroph-Pathotroph-Symbiotroph); Sap.-Sym. (Saprotroph-Symbiotroph).

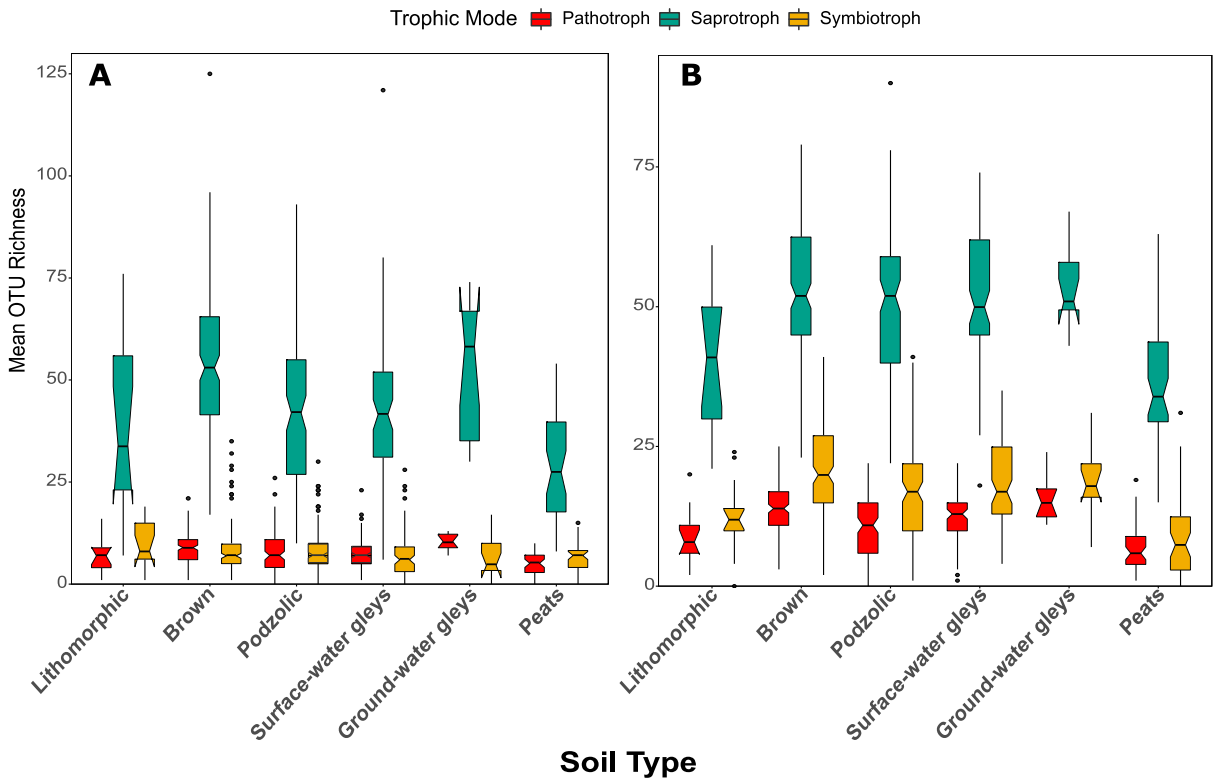

Fig. S10. Boxplots of richness of fungal OTUs matched to the pathotrophic, saprotroph, and symbiotroph trophic modes in FUNGuild for **A)** ITS1 and **B)** 18S datasets plotted against soil types. Soil types are listed in order of increasing approximate moisture content. Boxes cover the first and third quartiles and horizontal lines denote the median. Black dots represent outliers beyond the whiskers, which cover 1.5X the interquartile range.