Linking diagnostic features to soil microbial biomass and respiration in agricultural grassland soil: a large-scale study in Ireland

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Summary

The functional potential of soil ecosystems can be predicted from the activity and abundance of the microbial community in relation to key soil properties. When describing microbial community dynamics, soil physicochemical properties have traditionally been used. The extent of correlations between properties, however, differs between studies, especially across larger spatial scales. In this research we analysed soil microbial biomass and substrate-induced respiration of 156 samples from Irish grasslands. In addition to the standard physicochemical, soil type and land management variables, soil diagnostic properties were included to identify if these important soil–landscape genesis classes affected microbial biomass and respiration dynamics in Irish soil. Apart from physicochemical properties, soil drainage class was identified as having an important effect on microbial properties. In particular, biomass-specific basal (qCO2) and substrate-induced respiration (SIR:CFE) were explained best by the soil drainage. Poorly drained soil had smaller values of these respiration measures than well-drained soil. We concluded that this resulted from different groups within the microbial community that could use readily available carbon sources, which suggests a change in microbial community dynamics associated with soil texture and periods of water stress. Overall, our results indicate that soil quality assessments should include both physicochemical properties and diagnostic classes, to provide a better understanding of the behaviour of soil microbial communities.

Highlights:

• Assessing the effect of soil diagnostic features and properties on microbial biomass and respiration

• A soil biological survey from 156 grassland sites in Ireland

• Soil drainage class has an important effect on microbial properties

• Soil quality assessments should include both physicochemical properties and diagnostic classes

Introduction

Soil microbial communities play an important role in providing the majority of soil functions. Soil functions derived from soil microbial activities include: supporting services for primary production such as the mineralization and cycling of nutrients (Jones et al., 2009), and regulatory services that promote the biogenic stability of soil structure, which enhances water infiltration and root growth (Bronick & Lal, 2005), together with additional services that include the role of soil microbes as food resources for other species in the soil environment (Coleman, 2008). Because of the vital role that soil microbial communities play in ecosystem functioning, there is a need to assess and monitor soil in relation to soil quality and potential function.

Research on soil biodiversity and biological function over the last 30 years has focused predominantly on micro, plot and field-scale studies (Parkin, 1993). Large-scale studies have only recently become more common (Drenovsky et al., 2010; Dequiedt et al., 2011; Colman & Schimel, 2013). Research has shown that the effect of environmental, climatic and anthropogenic factors on
soil microbial properties depends on the heterogeneous nature of soil properties and their interactions across different spatial scales (Fierer et al., 2009; Colman & Schimel, 2013). For example, pH, organic carbon and moisture availability have important effects on microbial community structure and function (Hofman et al., 2004; Wakelin et al., 2008). However, the extent of the effects of these variables can also differ and vary with specific biomes and regions in relation to other variables such as plant diversity, climate and land management (Dequiedt et al., 2011; Colman & Schimel, 2013). Hofman et al. (2003) recommended that microbial indices, including biomass specific available carbon, biomass specific respiration, ratio of microbial biomass to organic matter and carbon availability index, should be included in large-scale soil and monitoring studies, together with microbial biomass, function and community structure. This enables the status of microbial communities in relation to differing environmental conditions to be analysed. There is still a need, however, to identify the ranges of these microbial indices in relation to biotic and abiotic factors and characteristic thresholds for specific soil categories, especially at medium and large biogeographical scales (Hofman et al., 2004; Dequiedt et al., 2011).

This study was part of a large soil survey by the Irish Soil Information System project between 2012 and 2013 (Creamer et al., 2014). In addition to the standard physical and chemical properties (Doran & Parkin, 1994), sampled soil was categorized by its diagnostic features. The diagnostic features describe conditions of the surface and subsurface horizon (A & B), which were expected to affect microbial abundance and function; these classes included drainage, organic matter, spodicity and argillicity. Drainage class indicates the natural state of drainage, which is affected by soil structure and water permeability. Poorly drained soil becomes saturated with water for extended periods, which creates an anaerobic environment. These conditions are expected to change and shape microbial community structures (Drenovsky et al., 2010). Organic matter quantity and quality are strongly associated with microbial biomass and function (Oberholzer et al., 1999); therefore, microbial properties are expected to differ between organic matter classes. Spodicity describes the loss of cations from the surface soil as a result of acidic conditions and presence of humic acids. Cations are essential micronutrients for microbes; iron in particular is required for electron transfer reactions in the respiratory pathway (Kirchman, 2012). Argillicity describes the translocation of clay minerals from an upper horizon to subsurface horizons, resulting in a reduction in clay minerals in the surface horizon and accumulation in the B horizon. Clay minerals are important in the development of soil structure (Bronick & Lal, 2005); they bind and concentrate organic matter, which reduces its availability as an energy supply for microbes (Strotzky, 1986). Clay has been positively correlated with microbial biomass (Dequiedt et al., 2011) and negatively with microbial respiration (Colman & Schimel, 2013).

Taking these categories and physicochemical properties into account, the aim of this study was to:

- identify the relationships of soil properties and diagnostic features with microbial biomass and microbial indices,
- assess the effect of diagnostic features, land use, soil types and soil properties on microbial respiration.

**Methods and materials**

**Sampling and sample preparation**

Sampling for soil properties was carried out at 156 geographically distinct sites throughout Ireland; these sites represented the full suite of soil series identified in the survey (non-probability sampling) (Figure 1) (Creamer et al., 2014). All sites were grasslands, which were grouped broadly into intensively managed (123 sites with inputs of lime, fertilizer and reseeding) and unmanaged (33 sites) grassland. Soil samples covered a broad range of soil types (Figure 1) and physicochemical properties (Table 1). Samples were collected over a 2-year period (2012–2013) from March to September, as part of a soil survey campaign. At each site, 1-m² profile pits were dug to facilitate description of soil types and sampling (following the Irish soil classification system) (Simó et al., 2014). Soil samples were taken from the cleaned surface horizon (horizon depths depended on local features and varied between 10 and 25-cm depth) using a sterile garden trowel. The amount of soil collected from each individual horizon was 2 kg for chemical and 1 kg for biological analysis. Soil for chemical analysis was dried at 40 °C for 72 hours and sieved to 2 mm. Soil for microbial analysis was stored at 4 °C for no longer than 1 week. Following sieving to 2 mm, soils were pre-incubated at 20 °C for 1 week prior to analysis to allow all samples to stabilize and standardize following sieving disturbance (Pell et al., 2006).

**Soil physical and chemical characteristics**

Methods used to describe soil physical and chemical characteristics followed protocols described in the Irish Soil Information System laboratory standard operational procedures manual (Massey et al., 2013). A full list of the soil physical and chemical characteristics that were evaluated is included in Table 1.

Soil texture was measured following the particle-size pipette method (ISO, 1998). Total nitrogen (N) and organic carbon (Corg) were determined by the LECO elemental analysis, following the milling and sieving of dried soil samples to 0.25 mm (ISO, 1995). Exchangeable cations, sodium (Na⁺), potassium (K⁺), magnesium (Mg²⁺), calcium (Ca²⁺) and effective cation exchange capacity (ECEC), were determined using the BaCl₂ extraction procedure adapted and modified from the ISO method (ISO, 1994). A field-based test that applied 10% HCl to the soil was used to assess for carbonates; this follows the FAO field guidelines (FAO, 2006). The pH was measured in a 1:2.5 (soil to solution ratio) suspension of soil in 0.01 m calcium chloride using a glass electrode (van Reeuwijk, 2002). Soil moisture (MC) was calculated from the difference between 5 g fresh soil and an oven-dried (24 hours at 105 °C) equivalent (van Reeuwijk, 2002). Oven-dried samples were used to determine organic matter (OM) by loss-on-ignition, adapted...
Figure 1 Sampling locations around Ireland marked by soil type (Irish Soil Classification System; WRB equivalents are: Peat (Histosol), Rendzina (Leptic Calcisol), Lithosols (Leptosol), Alluvial (Fluvisol), Groundwater Gleys (Gleysol), Surface-water Gleys (Stagnosols), Podzols (Podzols), Brown Podzolics (Entic Podzols), Luvisols (Luvisols), Brown Earths (Cambisols)).

from the British standard method (BS EN 13039, 2000). The samples were placed into a muffle furnace at 550°C for 16 hours and organic matter content calculated from the difference between pre-ash and ignited samples. The Haines-funnel system was used to measure the water-holding capacity (WHC) of the sample. A total of 100 ml of water was added to 50 g of fresh soil and left in a closed funnel. After 30 minutes the funnel was opened and excess water was collected from the system; its volume was measured and the
Table 1 Summary statistics of soil physicochemical and microbial properties measured in 156 soil samples throughout Ireland. If skewness was not acceptable, transformation by natural logarithm of the variable was applied. Skewness after ln-transformation is presented in the last column (NA: no transformation applied).

| Properties analysed                  | Mean   | Median | Min   | Max   | Standard deviation | Skewness | Skewness after ln-transformation |
|--------------------------------------|--------|--------|-------|-------|--------------------|----------|---------------------------------|
| Physical-chemical properties         |        |        |       |       |                    |          |                                 |
| N%                                   | 0.51   | 0.45   | 0.05  | 2.53  | 0.31               | 2.56     | 1.33                            |
| Corg%                                | 5.70   | 4.28   | 0.37  | 36.1  | 4.93               | 3.06     | 0.16                            |
| pH (CaCl₂)                           | 5.34   | 5.26   | 3.70  | 7.58  | 0.67               | 0.71     | NA                              |
| OM%                                  | 14.3   | 11.8   | 3.56  | 62.9  | 9.43               | 2.39     | 0.54                            |
| MC%                                  | 35.9   | 34.1   | 10.3  | 77.1  | 11.5               | 0.93     | NA                              |
| WHC/ml 100 g⁻¹                       | 78.8   | 78.1   | 46.5  | 129   | 14.9               | 0.70     | NA                              |
| Na⁺/cmol kg⁻¹                        | 0.17   | 0.12   | 0.04  | 0.77  | 0.13               | 2.22     | 1.86                            |
| K⁺/cmol kg⁻¹                         | 0.33   | 0.23   | 0.04  | 1.90  | 0.31               | 2.72     | 1.87                            |
| Mg²⁺/cmol kg⁻¹                       | 1.42   | 0.94   | 0.15  | 9.21  | 1.39               | 2.71     | 1.06                            |
| Ca²⁺/cmol kg⁻¹                       | 13.1   | 10.7   | 0.52  | 110   | 11.8               | 4.34     | 0.29                            |
| CEC/cmol kg⁻¹                        | 15.4   | 12.9   | 2.55  | 47.5  | 8.90               | 1.66     | 0.08                            |
| Sand%                                | 45.0   | 46.0   | 0.00  | 93.0  | 18.3               | −0.49    | NA                              |
| Clay%                                | 20.9   | 19.0   | 0.00  | 57.0  | 10.3               | 0.46     | NA                              |
| Microbial biomass properties         |        |        |       |       |                    |          |                                 |
| Cₘₐᵢₙ/µg C g⁻¹ dry soil              | 1711   | 1274   | 345.0 | 8584  | 1369               | 2.47     | 0.47                            |
| Nₘₐᵢₙ/µg N g⁻¹ dry soil              | 264    | 222    | 73.7  | 1213  | 154                | 2.47     | 0.33                            |
| Cₘₐᵢₙ:Nₘₐᵢₙ ratio                   | 6.23   | 5.62   | 2.02  | 16.3  | 2.23               | 1.57     | 0.46                            |
| Cₙₑₓ/µg g⁻¹ dry soil                 | 305    | 263    | 133   | 1422  | 165                | 3.89     | 1.18                            |
| Cₙₑₓ:Cₘₐᵢₙ/µg Cₙₑₓ mg⁻¹ Cₘₐᵢₙ      | 240    | 207    | 61.9  | 1017  | 147                | 1.82     | 0.09                            |
| Cₘₐᵢₙ:Cₙₑₓ%                         | 3.90   | 3.02   | 0.61  | 52.6  | 4.96               | 7.48     | 0.93                            |
| Microbial respiration properties     |        |        |       |       |                    |          |                                 |
| Water (BR)/µg CO₂-C g⁻¹ hour⁻¹       | 1.87   | 1.39   | 0.37  | 8.32  | 1.52               | 2.06     | 0.97                            |
| Gal/µg CO₂-C g⁻¹ hour⁻¹              | 3.35   | 2.87   | 0.95  | 11.2  | 1.89               | 1.52     | 0.49                            |
| MA/µg CO₂-C g⁻¹ hour⁻¹               | 5.33   | 4.46   | 1.44  | 14.7  | 2.56               | 1.24     | 0.20                            |
| GABA/µg CO₂-C g⁻¹ hour⁻¹             | 2.75   | 2.28   | 0.74  | 10.9  | 1.80               | 2.04     | 0.75                            |
| NAGA/µg CO₂-C g⁻¹ hour⁻¹             | 3.26   | 2.77   | 0.87  | 12.8  | 2.03               | 1.84     | 0.58                            |
| Glu (PR)/µg CO₂-C g⁻¹ hour⁻¹         | 4.91   | 4.33   | 1.43  | 14.5  | 2.51               | 1.09     | 0.21                            |
| AKGA/µg CO₂-C g⁻¹ hour⁻¹             | 5.90   | 5.43   | 1.20  | 17.3  | 2.80               | 1.09     | −0.10                           |
| CA/µg CO₂-C g⁻¹ hour⁻¹               | 4.35   | 3.82   | 0.99  | 16.6  | 2.45               | 1.97     | 0.28                            |
| qCO₂-Water/µg CO₂-C mg⁻¹ Cₘₐᵢₙ hour⁻¹ | 1.34   | 1.07   | 0.23  | 6.18  | 0.98               | 1.76     | 0.69                            |
| qCO₂-Glucose/µg CO₂-C mg⁻¹ Cₘₐᵢₙ hour⁻¹ | 3.54   | 3.12   | 0.81  | 10.2  | 1.65               | 1.02     | 0.11                            |
| CAI (PR/BR)                          | 3.25   | 3.25   | 0.99  | 6.57  | 1.29               | 0.37     | NA                              |

BR, basal respiration; Gal, galactose; MA, malic acid; GABA, γ-aminobutyric acid; NAGA, n-acetyl glucosamine; Glu, glucose; AKGA, α-ketoglutaric acid; CA, citric acid.

The amount of water retained in the soil calculated (ml water 100 g⁻¹ soil) (Jenkinson & Powlson, 1976).

Diagnostic features

The concept of diagnostic features was originally developed in the World Reference Base (WRB) classification system (FAO, 2006), where recognition of diagnostic materials and features (for example gleic properties) can be used to explain the main soil-forming and anthropogenically-influenced processes in soil. The inclusion of diagnostic features is seen as part of the wider classification system, identifying the presence of key genetic horizons (Láng et al., 2013; Schulte et al., 2015). Four diagnostic features, soil drainage, organic matter status, loss of cations from the surface horizon through leaching (spodicity) and deposition of clay down the soil profile by illuviation (argillicity), were selected in this study as potential controlling factors. Table 2 describes the diagnostic features taken into account in this study.

Soil microbial analysis

Two indicators (microbial biomass and multiple substrate-induced respiration) were chosen to describe the main microbial properties within soil. Microbial biomass is an indicator of the total microbial abundance of the soil (Vance et al., 1987), whereas multiple substrate-induced respiration reflects the potential microbial activity (Campbell et al., 2003).

Microbial biomass. Microbial biomass was measured by the chloroform fumigation extraction method (CFE) (Vance et al., 1987). Ten grams dry weight equivalent for each soil were fumigated with chloroform for 24 hours. Soluble organic carbon and nitrogen...
Table 2 Identification of soil diagnostic categories

| Organic matter class | Drainage class | Argillicity class | Spodicity class |
|----------------------|---------------|-------------------|-----------------|
| Mineral              | 0–3.5% OC     | No evidence of gleying | Typical         |
| Humic                | 3.5–12% OC    | Evidence of gleying between 40 and 80 cm of soil profile | Argic           |
| Histic               | >12% OC       | Clear evidence of gleying within 40 cm of soil profile | Luvic           |
|                      |               |                   | Spodic          |

were extracted from fumigated and unfumigated samples with 0.5 M K_2SO_4 (1:4 soil:solution ratio). After adding the reagent, samples were shaken for 30 minutes on a side-to-side shaker and extracts were filtered. Total organic carbon and nitrogen were determined using a Shimadzu (Kyoto, Japan) TOC-TN analyser. Microbial biomass carbon (C_mic) and nitrogen (N_mic) were calculated from the difference between fumigated and unfumigated samples using a conversion factor (EC) of 0.45 (Solaiman, 2007). Carbon extracted from unfumigated samples was used as a measure of extractable extracellular carbon (C_ext) (Hofman et al., 2004).

**Multiple substrate-induced respiration assay.** Potential substrate-induced respiration (PR) and basal respiration (BR) were measured by the MicroResp™ method. This community level physiological profiling (CLPP) method applies a microtiter plate design to assess catabolic activity of whole soil samples (Campbell et al., 2003). Water and a spectrum of seven carbon substrates, including D-galactose (Gal), D-glucose (Glu), L-malic acid (MA), α-ketoglutaric acid (AKGA), citric acid (CA), γ-aminobutyric acid (GABA) and n-acetyl glucosamine (NAGA), were used. These substrates were chosen from 15 suggested substrates given in Campbell et al. (2003) and cover a range of carbohydrates, carboxylic as well as amino acids and one amide. This relatively small set of substrates has been shown to discriminate successfully between soils in other studies (Creamer et al., 2009; Creamer et al., 2015). It also allows for a robust replication for each carbon source (12 replicates) per 96-well multi-plate system. Substrates were prepared to a concentration of 30 mg g⁻¹ soil water and 25-μl aliquots were dispensed randomly into each well of a 96-deep-well microtiter plate (12 wells per substrate). Approximately 30 mg of soil (corrected to a water-holding capacity of between 30 and 60%) was dispensed into each well in the MicroResp™ plate system, using a filling device. The plates were left to rest for 30 minutes in the dark before sealing to avoid detecting CO₂ emissions released by chemical reactions other than respiration in the soil (Creamer et al., 2009). Absorbance of the colorimetric gel detector plate was read at 570 nm (Modulus™ Microplate Multimode Reader, Turner BioSystems, Inc. 2007, Sunnyvale, CA, USA) before incubation, and the plate was then sealed to the deep-well plate. The indicator plate was prepared 7 days in advance and consisted of cresol red (12.5 μl l⁻¹), potassium chloride (150 mM) and sodium bicarbonate (2.5 mM), all set in 150 μl purified agar (1%). Finally, the MicroResp™ unit was closed tightly with two metal clamps and incubated at 25 °C for 6 hours. Absorbance (A_570) of the gel detector plate was read a second time after 6 hours and respiration rates were calculated (μg CO₂-C g⁻¹ hour⁻¹) from an independent calibration curve (as described by Creamer et al., 2009). The calibration curve was generated using a plate reader (Modulus™ Microplate Multimode Reader, Turner BioSystems, Inc. 2007) and gas chromatograph (Varian CP–3800 GC, Walnut Creek, CA, USA). Absorbance and CO₂ were determined from incubation microcosms within small glass jars, which were sealed with rubber bungs and septa. Six different soils were added to the jars in 10-, 20- and 30-g aliquots (three replicates each). The soil microcosms were then amended with either water or glucose solutions (30 mg g⁻¹ soil water). An eight-well strip of a 96-well strip plate (prepared like the indicator plate) was then added before the jars were sealed. Before and after 6 hours of incubation at 25 °C, absorbance was measured (A_570) and CO₂ was collected from the headspace of the sealed microcosms using gas-tight syringes. Gases were stored in evacuated vials until measured on the gas chromatograph. Generated data were fitted by a curve using non-linear regression analysis performed with the Statistica 10 package (StatSoft Inc., 2010, Tulsa, OK, USA). The best fitting calibration curve was as follows:

\[
\text{percentage of } \text{CO}_2 = A + B/(1 + D \times A_{570})
\]

where A was −0.16, B was −0.48 and D was −4.16.

**Calculation of microbial indices.** Determination of microbial biomass and multiple substrate-induced respiration facilitated calculation of the following microbial indices (Hofman et al., 2004):

- biomass-specific basal respiration quotient (qCO₂) and biomass-specific substrate-induced respiration for each substrate (SIR:CFE) (μg CO₂–C mg⁻¹ C_mic hour⁻¹);
- carbon availability index (CAI = PR_Glucose:BR);
• biomass-specific available organic carbon $C_{\text{ext}}$:C$_{\text{mic}}$ ratio (µg $C_{\text{ext}}$ mg$^{-1}$ C$_{\text{mic}}$); and
• microbial coefficient C$_{\text{mic}}$:C$_{\text{org}}$ (%).

Statistical analysis

Assessment of diagnostic categories, land-use and soil types. For all analyses, variables were transformed to natural logarithms (ln) to reduce skewness in the data when necessary (skewness values larger than 1 or smaller than −1; Table 1). We checked the homogeneity of variances using Levene’s test and normality of the residuals visually by inspecting histograms of residual frequencies. To evaluate statistical relations of diagnostic features, land use and soil types with microbial biomass and respiration indices, we used a one-way analysis of variance (ANOVA) in a model-based approach. By taking a model-based approach, we implicitly assumed a statistical model that treats the dependent variable (i.e. the log-transformed) microbial biomass and respiration indices) as the sum of a deterministic mean (a function of the explanatory variables, i.e. the diagnostic features, land use and soil types) and a zero-mean, independently, identically and normally distributed stochastic residual. Thus, independence follows from the model assumptions and need not be achieved through random sampling. To identify pairwise differences between diagnostic classes, Fisher’s least significant difference (LSD) post hoc test was used. Confidence intervals of group means were also determined by bootstrapping with 1000 re-samplings. We assumed that for all locations in the study area the assumptions of a linear model held, enabling inferences based on correlation about grassland soils in Ireland (Snedecor & Cochran, 1989).

Environmental factors related to microbial biomass and microbial indices. Correlations between all measured physicochemical and microbial variables were calculated using Pearson’s correlation coefficient (Table 3). In addition, individual multiple regression analyses were performed to select significant predictor variables of microbial properties. All diagnostic and physicochemical properties were included as predictors. Individual dummy variables were created for the diagnostic classes (two for each because they are complementary within a diagnostic feature, land-use and soil type factors. Forward stepwise selection was used and the most suitable models were chosen on the basis of the Akaike information criterion (the model with the smallest AIC represents the best model; ΔAIC < 2 was taken as the threshold value for improvement). Multi-collinearities in the best fitting models were checked by calculating variance inflation factors; no values larger than four were found, indicating that no problematic collinearities were present.

Environmental factors related to multiple substrate-induced respiration

Changes in multiple substrate-induced respiration (hereafter referred to as microresp) and respiration per unit biomass (gCO$_2$ and SIR:CFE) in relation to physicochemical properties, diagnostic classes, land-use and soil type were analysed by redundancy analysis (RDA). This method extracts and summarizes the variation in a set of response variables that can be explained by a set of explanatory variables (Borcard et al., 2011). The axes defined in the space of the explanatory variables are orthogonal to one another. The matrix of explanatory variables conditions the eigenvalues, and the orthogonality and direction of the ordination axes. In short, each response variable is regressed on all explanatory variables, and fitted values and residuals are calculated. A matrix filled with unstandardized fitted response variables is then subjected to principal components analysis (PCA). Subsequently, correlations between the explanatory variables and the ordination axes are calculated. Lists of explanatory and response variables included in the RDA analyses are given in Table 5. Two separate RDAs were applied: one using the log-transformed respiration data from all carbon sources, the second one using the log-transformed specific respiration data (divided by microbial biomass). Environmental variables were tested for inclusion in the RDA model by forward step-wise selection based on 499 Monte Carlo permutations. A final RDA model including only the significant variables was then run to test the significance of all canonical axes. Analysis of variance and regression analyses were carried out with SPSS 22.0.0 (IBM Corp, Armonk, NY, USA). Redundancy analysis was performed using CANOCO 5 (Biometris, 2014, Plant Research International, Wageningen, the Netherlands).

Results

Assessment of diagnostic categories, land-use and soil types

Individual ANOVA tests were used to investigate pairwise effects of diagnostic features, land management and soil type on ln-transformed microbial biomass and respiration properties (Tables S1–S6, Supporting Information).

Microbial C and N, and the ratio C$_{\text{mic}}$:N$_{\text{mic}}$ are significantly different between OM classes, with a larger value in histic than in mineral soils (respectively, $F = 27.8$, $P < 0.001$; $F = 12.0$, $P < 0.001$; $F = 21.6$, $P < 0.001$; Figure 2a,b,c). The overall respiration (microresp) is larger for all substrates for histic than mineral soils ($P < 0.001$) (Figure 3a). Analysis of SIR:CFE showed that water and three substrates were not significantly different between OM classes; however, the use of MA, AKGA, CA and glucose resulted in a larger ($P < 0.05$) SIR:CFE for mineral than for histic soils (Figure 3b). Mean values of C$_{\text{ext}}$ are larger for histic than mineral soils ($F = 11.2$, $P < 0.001$; Figure 4a). This is in contrast to the C$_{\text{ext}}$-C$_{\text{mic}}$ ratio and CAI, which have a smaller mean for histic than for mineral soils ($F = 12.9$, $P < 0.001$; Figure 4b, and $F = 3.68$, $P = 0.028$; Figure 4c, respectively).

The mean C$_{\text{mic}}$ ($P < 0.001$) and C$_{\text{mic}}$:N$_{\text{mic}}$ ($P < 0.001$) are significantly larger for poorly drained soil (Figure 5a,b). There are no differences between drainage classes for C$_{\text{ext}}$ and C$_{\text{mic}}$:C$_{\text{ext}}$; but C$_{\text{ext}}$:C$_{\text{mic}}$ values are significantly smaller for poorly drained than for well and moderately drained soils (Figure 5c). Mean SIR:CFE values are also smaller ($P < 0.01$) for all substrates in poorly drained when compared with well-drained soils (Figure 6).
Table 3 Pearson’s correlation coefficient (r) for each bivariate correlation of physicochemical and microbial properties. Correlations were tested on ln-transformed properties (except for pH, MC, WHC, sand and clay, which were not transformed)

|       | N  | Corg | pH   | OM   | MC   | WHC | Na⁺   | K⁺   | Mg²⁺ | Ca²⁺ | CEC   | Sand | Clay | Cmic | Nmic | Cmic*Nmic | Cext | Ceext/Cmic | Cmic/Corg | qCO₂-Water | qCO₂-Glucose |
|-------|----|------|------|------|------|-----|-------|------|------|------|-------|------|------|------|------|-----------|------|------------|-----------|------------|--------------|
| Corg  | 0.99 |      |      |      |      |     |       |      |      |      |       |      |      |      |      |           |      |            |            |            |              |
| pH    | −0.06 | −0.07 |      |      |      |     |       |      |      |      |       |      |      |      |      |           |      |            |            |            |              |
| OM    | 0.79 | 0.77 | −0.14 |      |      |     |       |      |      |      |       |      |      |      |      |           |      |            |            |            |              |
| MC    | 0.70 | 0.68 | −0.21 | 0.76 |      |     |       |      |      |      |       |      |      |      |      |           |      |            |            |            |              |
| WHC   | 0.76 | 0.76 | −0.19 | 0.76 | 0.69 |     |       |      |      |      |       |      |      |      |      |           |      |            |            |            |              |
| Na⁺   | 0.37 | 0.37 | −0.15 | 0.35 | 0.37 | 0.25 |       |      |      |      |       |      |      |      |      |           |      |            |            |            |              |
| K⁺    | 0.32 | 0.33 | −0.01 | 0.21 | 0.18 | 0.27 | 0.24  |      |      |      |       |      |      |      |      |           |      |            |            |            |              |
| Mg²⁺  | 0.42 | 0.41 | 0.00  | 0.24 | 0.37 | 0.33 | 0.39  | 0.39 |      |      |       |      |      |      |      |           |      |            |            |            |              |
| Ca²⁺  | 0.48 | 0.46 | 0.52  | 0.38 | 0.24 | 0.28 | 0.05  | 0.22 | 0.29 |      |       |      |      |      |      |           |      |            |            |            |              |
| CEC   | 0.59 | 0.58 | 0.41  | 0.49 | 0.38 | 0.43 | 0.23  | 0.30 | 0.47 | 0.89 |      |      |      |      |      |           |      |            |            |            |              |
| Sand  | −0.47 | −0.45 | 0.06  | −0.48 | −0.40 | −0.24 | −0.33 | −0.39 | −0.40 |      |      |      |      |      |      |           |      |            |            |            |              |
| Clay  | 0.09 | 0.11 | 0.03  | 0.06 | −0.05 | 0.21 | −0.03 | 0.26 | 0.03 | 0.18 | 0.17 | −0.42 |      |      |      |           |      |            |            |            |              |
| Cmic  | 0.69 | 0.68 | −0.25 | 0.71 | 0.65 | 0.72 | 0.33  | 0.35 | 0.31 | 0.19 | 0.34 | −0.41 | 0.18 |      |      |           |      |            |            |            |              |
| Nmic  | 0.61 | 0.61 | −0.04 | 0.56 | 0.52 | 0.62 | 0.24  | 0.30 | 0.26 | 0.19 | 0.30 | −0.35 | 0.15 | 0.87 |      |           |      |            |            |            |              |
| Cmic*Nmic | 0.44 | 0.44 | −0.33 | 0.55 | 0.50 | 0.48 | 0.26  | 0.24 | 0.19 | 0.15 | 0.29 | −0.25 | 0.14 | 0.65 | 0.22 |           |      |            |            |            |              |
| Cext  | 0.58 | 0.56 | 0.01  | 0.41 | 0.39 | 0.51 | 0.15  | 0.09 | 0.26 | 0.16 | 0.25 | −0.29 | 0.00 | 0.49 | 0.59 | 0.12      |      |            |            |            |              |
| Ceext/Cmic | −0.39 | −0.40 | 0.29 | −0.53 | −0.48 | −0.48 | −0.27 | −0.33 | −0.17 | −0.11 | −0.22 | 0.27 | −0.20 | −0.30 | −0.39 | −0.66 | 0.12      |      |            |            |            |              |
| Cmic/Corg | 0.57 | 0.55 | 0.01  | 0.40 | 0.38 | 0.51 | 0.15  | 0.09 | 0.25 | 0.15 | 0.25 | −0.28 | 0.01 | 0.49 | 0.58 | 0.12      | 1.00 | 0.12      |            |            |              |
| qCO₂-Water | −0.06 | −0.06 | 0.04 | −0.11 | 0.08 | 0.01 | −0.09 | −0.19 | 0.14 | 0.02 | −0.01 | 0.07 | −0.10 | −0.38 | −0.36 | −0.21 | −0.03 | 0.41   | −0.03      |            |            |              |
| qCO₂-Glucose | −0.24 | −0.23 | 0.20 | −0.19 | −0.06 | −0.18 | −0.15 | −0.17 | −0.02 | −0.01 | −0.07 | 0.16 | −0.16 | −0.57 | −0.44 | −0.43 | −0.19 | 0.51   | −0.19 | 0.77      |            |            |              |
| CAI   | −0.12 | −0.10 | 0.08 | 0.00 | −0.14 | −0.07 | −0.08 | 0.17 | −0.25 | −0.08 | −0.06 | 0.03 | 0.09 | 0.15 | −0.05 | −0.17 | −0.22 | −0.17 | −0.66 | −0.19 |           |            |            |              |

Corg, organic carbon; OM, organic matter; MC, moisture content; WHC, water holding capacity; Na⁺, sodium; K⁺, potassium; Mg²⁺, magnesium; Ca²⁺, calcium; CEC, cation exchange capacity; Cmic, microbial biomass carbon; Nmic, microbial nitrogen; Ceext, extractable extracellular carbon; qCO₂, respiration quotient; CAI, carbon availability index.
Factors affecting soil microbial biomass and respiration

**Figure 2** Box and whisker plots of ln-transformed (a) microbial biomass carbon ($C_{mic}$ in mg C g$^{-1}$ soil), (b) microbial biomass nitrogen ($N_{mic}$ in mg N g$^{-1}$ soil) and (c) $C_{mic}:N_{mic}$ ratio (in mg C mg$^{-1}$ N). Data are shown for each diagnostic organic matter class: mineral ($n=94$), humic ($n=52$) and histic ($n=10$) soil samples. Boxes display lower and upper quartiles and median. Whiskers show 1.5 × interquartile range.

**Figure 3** Box and whisker plots of ln-transformed (a) respiration (in µg CO$_2$-C g$^{-1}$ soil hour$^{-1}$) and (b) respiration per unit biomass (in µg CO$_2$-C mg$^{-1}$ C$_{mic}$ hour$^{-1}$) for substrates water, γ-aminobutyric acid (GABA), α-acetyl glucosamine (NAGA), D-galactose (Gal), citric acid (CA), D-glucose (Glu), L-malic acid (MA) and α-ketoglutaric acid (AKGA). Respiration data for each substrate are shown for each diagnostic organic matter class: mineral ($n=94$), humic ($n=52$) and histic ($n=10$). Boxes display lower and upper quartiles and median. Whiskers show 1.5 × interquartile range.

**Figure 4** Box and whisker plots of ln-transformed (a) biomass available carbon ($C_{ext}$ in µg g$^{-1}$ soil), (b) $C_{ext}:C_{mic}$ ratio (in µg C$_{ext}$ mg$^{-1}$ C$_{mic}$) and (c) carbon availability index (CAI). Data are shown for each diagnostic organic matter class: mineral ($n=94$), humic ($n=52$) and histic ($n=10$). Boxes display lower and upper quartiles and median. Whiskers show 1.5 × interquartile range.
Well Moderate Poor
ln(Cmic/(µg C g⁻¹ soil))

Well Moderate Poor
ln(Cmic:Nmic/(µg C µg⁻¹ N))

Well Moderate Poor
ln(Cext:Cmic/(µg Cext mg⁻¹ Cmic))

Figure 5 Box and whisker plots of ln-transformed (a) microbial biomass carbon (Cmic in mg C g⁻¹ soil), (b) microbial biomass carbon and nitrogen ratio (Cmic:Nmic in mg C mg⁻¹ N) and (c) biomass specific available carbon (Cext:Cmic ratio in µg Cext mg⁻¹ Cmic). Data are shown for each diagnostic drainage class: well drained (n = 74), moderately drained (n = 36) and poorly drained (n = 46). Boxes display lower and upper quartiles and median. Whiskers show 1.5 x interquartile range.

Although significant differences were found in some cases, there were no strong effects on microbial properties for soil type, land management, argillicity and spodicity.

Environmental factors related to microbial biomass and microbial indices

Models were generated by multivariate regression analysis that explained from 17 (CAI) to 66% (Cmic) of the variances within microbial properties. Physicochemical properties, especially OM, WHC, N and pH (Table 4), are shown as significant predictors. Organic matter content, in particular, explained the most variation within Cmic:Nmic (30%) and Cext:Cmic (28%). WHC was the best predictor for Cmic (52%) and Nmic (38%), and N was the best predictor for Cext (33%) and Cmic:Corg (32%). Argillicity affected Cmic:Corg and CAI, whereas drainage class explained a significant proportion of the variation for Cmic, Cmic:Nmic, Cext and Cext:Cmic. Organic matter content and drainage class were tested further for interaction, but regression models revealed no interaction between the two variables in relation to Cmic:Nmic and Cext:Cmic. Microbial C, however, shows a steeper response with increasing OM in poorly drained soil when compared with moderately and well-drained soils (Figure 7).

Environmental factors related to multiple substrate-induced respiration

Redundancy analysis revealed that the variation in microresp data was described most thoroughly by soil properties N, pH, OM, Mg²⁺, Cu²⁺ and diagnostic drainage class. A total variation of 44% was explained by this model (P < 0.05), and N was the most prominent predictor. The two-dimensional redundancy plot (Figure 8) of respiration measures shows that the RD1 axis (explaining 39% variation) corresponds to the increase in overall respiration (all substrates showed positive values) and is positively related to N, OM and Mg²⁺. The second RDA axis explains the differences between the C substrate utilization (as scores were similar on the first axis, but differed on the second axis), and is related to pH and drainage class. Nevertheless, only 4% variance is explained by the RD2 axis.

Redundancy analysis of qCO₂ and SIR:CFE data explains 38% of the total sample variance (P < 0.05), and drainage class is the most prominent predictor. Again, the two-dimensional redundancy plot (Figure 9) shows that RD1 represents the gradient of qCO₂ and SIR:CFE yield, whereas RD2 relates to the difference in SIR:CFE.
between the carboxylic acids and all other substrates. Potassium and N show a negative effect in relation to qCO₂ and SIR:CFE yield, whereas pH is positively related to the SIR:CFE ratio of MA, AKGA and CA. Poorly drained soils produce the smallest values of qCO₂ and SIR:CFE.

### Discussion

*Important physicochemical properties in Irish grassland soil*

The functional potential of soil ecosystems can be predicted from the activity and abundance of the microbial community.
indices and total respiration. In this paper, these physicochemical properties for microbial biomass, microbial respiration, an unknown proportion of the CO$_2$ measured from OM and N in relation to respiration and microbial biomass is well spanning a range of physicochemical properties. Hofman et al., 2008; Creamer et al., 2015) found that soil organic carbon had the strongest correlation with microbial biomass, when comparing 400 soils across different biomes. Hofman et al., 2004 concluded that OM and N were key properties affecting microbial biomass and respiration of 117 soils collected in the Czech Republic and spanning a range of physicochemical properties.

The pH has also been shown to be a controlling factor for differences in carbon utilization patterns (Wakelin et al., 2008; Creamer et al., 2015). Although pH was a significant predictor for C$_{mic}$, the C$_{mic}$:N$_{mic}$ and C$_{ext}$:C$_{mic}$ ratios, in this study we identified only a marginally significant effect of this variable in relation to differences in respiration patterns. The pH was associated with increased respiration rates of the carboxylic acids AKGA and CA for microresp, as well as increased SIR-CFE for MA, AKGA and CA. Enzymes related to the catabolism of carbon substrates are pH sensitive (Tutu & Ciornea, 2011), but differences in respiration values between soils with the highest and lowest pH were very large. Therefore, despite using an adapted microresp method, which aimed to reduce detection of soil chemical CO$_2$ fluxes not related to microbial respiration, an unknown proportion of the CO$_2$ measured may have resulted from the release of CO$_2$ from bicarbonate pools in soils (Martens, 1987).

Finally in this paper, potassium was closely correlated with C$_{mic}$, C$_{mic}$:N$_{mic}$, C$_{ext}$:C$_{mic}$ and CAI, whereas magnesium was correlated with microbial respiration. Both cations were significant in explaining variations in CAI and respiration per unit biomass. Their correlation with microbial properties is not surprising because K is required for microbial growth and the K$^+$/Mg$^{2+}$ ratio is important for protein synthesis (Tempest et al., 1966).

**Importance of soil diagnostic classes**

In addition to the physicochemical properties described, this study showed that grouping soils into diagnostic OM classes (mineral, humic and histic) explained clear trends in microbial biomass and respiration that could be related back to the microbial status of the soils within each category. Extracellular C (C$_{ext}$) has been associated with the readily mineralizable carbon fraction for the microbial community within soil (Hofman et al., 2003). Even though C$_{ext}$ was largest in histic soils, its proportion to C$_{mic}$ decreased from mineral to humic to histic soils in this study. This would indicate that more readily mineralizable carbon is fixed within the microbial biomass or as recalcitrant soil organic carbon. In soil samples defined as humic and histic, the carbon fixation seemed to be more evenly distributed between C$_{mic}$ and C$_{org}$, therefore, it did not change the C$_{mic}$:C$_{org}$ ratio compared with mineral soils. However, histic soils were distinguished from mineral soils in relation to CAI. The narrower carbon availability index for histic soils indicated a reduced demand to catabolize added carbon substrates than for mineral soils. The microbial community in histic soils showed less response in catabolizing simple added carbon substrates than in mineral and humic soils. By analysing the respiratory quotient, assumptions can be made in relation to the carbon demands for maintenance of the total biomass. Colman & Schimel (2013) suggested that soils with larger C pools have more labile C but slower basal respiration per unit biomass. The theory behind this is that soil communities can adapt to a more efficient use of energy and therefore can support a large biomass with smaller carbon demand (Anderson & Domsch, 2010). For this current study, there was no difference in qCO$_2$ between mineral, humic and histic soils. Nevertheless, addition of carboxylic acids resulted in significantly smaller SIR-CFE ratios for histic soils.

Although the predictive power of the more traditional physicochemical variables was superior in describing microbial biomass and total respiration across the whole dataset, these variables were less important when describing the behaviour of the whole community response in relation to basal and substrate-induced respiration per unit biomass. A total of 38% of the variation was explained by the variables in the current study, with drainage class being the most prominent predictor. This effect was related to smaller qCO$_2$ and SIR-CFE ratios for poorly drained soils than for well-drained soils and was found across all substrates used. Total C$_{mic}$ increased from well to poorly drained soils. This increase did not affect the C$_{mic}$:C$_{org}$ ratio because C$_{org}$ also increased from well to poorly drained soils. This was in contrast to the proportion of C$_{ext}$ and N$_{mic}$ to C$_{mic}$, which were both smaller in poorly drained...
Factors affecting soil microbial biomass and respiration

Table 5 List of explanatory (used in both analyses) and response variables used in first (results presented in Figure 8) and second RDA analyses (results presented in Figure 9). For diagnostic properties (n – 1) dummy variables were created (classes within a diagnostic property are linearly dependent, hence one category is dropped).

| Explanatory variables | Response variables 1/μg CO₂-C g⁻¹ hour⁻¹ | Response variables 2/μg CO₂-C mg⁻¹ Cmic hour⁻¹ |
|-----------------------|---------------------------------------------|---------------------------------------------|
| ln(N%)                | ln(Water)                                   | ln(Water (specific))                         |
| ln(Corg/%)            | ln(Water)                                   | ln(Water (specific))                         |
| pH(CaCl₂)             | ln(MA)                                      | ln(MA (specific))                            |
| ln(OM/%)              | ln(GABA)                                    | ln(GABA (specific))                          |
| ln(Na⁺/cmol kg⁻¹)     | ln(NAGA)                                    | ln(NAGA (specific))                          |
| ln(K⁺/cmol kg⁻¹)      | ln(Glu)                                     | ln(Glu (specific))                           |
| ln(Ca²⁺/cmol kg⁻¹)    | ln(AKGA)                                    | ln(AKGA (specific))                          |
| Sand/ %               | ln(CA)                                      | ln(CA (specific))                            |
| Clay/ %               |                                             |                                             |
| Drainage class – Well|                                             |                                             |
| Drainage class – Poor |                                             |                                             |
| Argillicity – Argic  |                                             |                                             |
| Argillicity – Luvic  |                                             |                                             |
| Spodicity – Spodic    |                                             |                                             |
| Spodicity – Podzolic  |                                             |                                             |

Gal, galactose; MA, malic acid; GABA, γ-aminobutyric acid; NAGA, n-acetyl glucosamine; Glu, glucose; AKGA, α-ketoglutaric acid; CA, citric acid.

Figure 8 Redundancy analysis of fitted regression values of respiration data (microresp) on environmental variables shown in the plane of the first two redundancy axes, RD1 and RD2. Variables N, drainage class, OM, Mg²⁺, pH and Ca²⁺ were included based on significant improvements (P < 0.05) in explaining the model variance. Explained variance for RD1 was 39% and for RD2 was 4%. Carbon substrates and water used in the MicroResp™ assay: D-galactose (Gal), D-glucose (Glu), L-malic acid (MA), α-ketoglutaric acid (AKGA), citric acid (CA), γ-aminobutyric acid (GABA) and n-acetyl glucosamine (NAGA).

Figure 9 Redundancy analysis of fitted regression values of all respiration per unit biomass data (qCO₂ and SIR:CFE) on environmental variables shown in the plane of the first two redundancy axes. Drainage class, pH, N, K⁺ and Mg²⁺ were selected to be significant (P < 0.05) in explaining model variance. Explained variance for RD1 was 35% and for RD2 was 2%. Carbon substrates and water used in the MicroResp™ assay: D-galactose (Gal), D-glucose (Glu), L-malic acid (MA), α-ketoglutaric acid (AKGA), citric acid (CA), γ-aminobutyric acid (GABA) and n-acetyl glucosamine (NAGA).
soils. The poorly drained soils analysed in this study were to a large extent fine textured (stagnogleys) and have been shown to support larger biomass because of adhesion and protection of microbes by clay particles (Mills, 2003). Multiple substrate-induced respiration methods like MicroResp™ measure the activity of microbes that respond quickly to added easily-degradable carbon sources. Results from the current study indicate that these microbes were present in a relatively smaller proportion of the total microbial community in poorly drained soils. Prolonged and recurring periods of waterlogging can have a significant influence on the microbial community structure (Drenovsky et al., 2010). In waterlogged soil most of the soil pore space is filled with water, causing a shift towards a larger proportional abundance of anaerobic and facultative anaerobic microbial species in relation to fast metabolizing aerobic microbial species.

Many studies have identified strong differences in microbial properties between land management types in agricultural soils (Grayston et al., 2001; Kaschuk et al., 2010). In this study, however, land management did not have a significant effect on the microbial biomass, microbial indices or respiration. Although larger average microbial biomass and activity were measured in unmanaged grassland soils compared with managed grassland soils, samples from both land management types had similar broad ranges in these microbial properties and results were therefore not statistically significant. The current study was completed as part of a national soil survey and was therefore restricted to specific time frames (sampling was throughout the growing season over a 2-year period). Therefore, this study did not account for temporal fluctuations in microbial biomass and respiration, previously related to differences in soil moisture, temperature and different stages of vegetation growth (Geisseler & Horwath, 2009). Temporal effects on microbial properties have been shown to differ between studies. For microbial biomass no strong inter-annual and seasonal differences were detected in a German regional study (Wirth, 2001) and temperate upland grassland study in the UK (Grayston et al., 2001), respectively. However, studies by Wirth (2001) and Grayston et al. (2001) did report significant responses between sampling years for microbial respiration, with the largest results recorded in spring. Zeller et al. (2001) showed that temporal fluctuations in microbial biomass were not consistent between alpine grassland sites and this reduced the effect of management. However, a French national soil survey carried out over several years by Dequiedt et al. (2011) did find differences between grassland management in relation to microbial biomass. Therefore, in this paper, we can only conclude that temporal fluctuations might have masked the effect of land management in our study, but this effect was less pronounced in relation to the physicochemical properties described and soil drainage capacity.

Conclusion

The research described above shows that apart from physicochemical soil properties, additional information related to pedogenetic processes (defined by diagnostic classes) in soil can be an important factor when considering the differences in microbial biomass and the physiological state of microbial communities across large sampling scales. The inclusion of diagnostic information, in particular soil drainage capacity, was important in understanding the variation in soil respiration not accounted for by the physicochemical properties alone. Poorly drained soils were defined by smaller respiration values than those for well-drained soils. We conclude that this results from a change in the microbial community composition, from an aerobic dynamic community to a more anaerobic and facultative anaerobic microbial system, associated with soil texture and periods of water stress.

Supporting Information

The following supporting information is available in the online version of this article:

Table S1 Effect of OM class on microbial biomass and respiration, tested using ANOVA.
Table S2 Effect of drainage class on microbial biomass and respiration, tested using ANOVA.
Table S3 Effect of soil type on microbial biomass and respiration, tested using ANOVA.
Table S4 Effect of land management on microbial biomass and respiration, tested using ANOVA.
Table S5 Effect of argillicity on microbial biomass and respiration, tested using ANOVA.
Table S6 Effect of spodicity on microbial biomass and respiration, tested using ANOVA.

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