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Soil Greenhouse Gas Fluxes, Environmental Controls, and the Partitioning of N$_2$O Sources in UK Natural and Seminatural Land Use Types

**Fotis Sgouridis** and **Sami Ullah**

1School of Geographical Sciences, University of Bristol, Bristol, UK, 2School of Geography, Earth and Environmental Sciences, and Birmingham Institute for Forest Research, University of Birmingham, Birmingham, UK

**Abstract** Natural and seminatural terrestrial ecosystems (unmanaged peatlands and forests and extensive and intensive grasslands) have been under-represented in the UK greenhouse gas (GHG) inventory. Mechanistic studies of GHG fluxes and their controls can improve the prediction of the currently uncertain GHG annual emission estimates. The source apportionment of N$_2$O emissions can further inform management plans for GHG mitigation. We have measured in situ GHG fluxes monthly in two replicated UK catchments and evaluated their environmental controlling factors. An adapted $^{15}$N-gas flux method with low addition of $^{15}$N tracer ($0.03$–$0.5$ kg $^{15}$N ha$^{-1}$) was used to quantify the relative contribution of denitrification to net N$_2$O production. Total N$_2$O fluxes were 40 times higher in the intensive grasslands than in the peatlands (range: $-1.32$ to $312.3 \mu$g N m$^{-2}$ h$^{-1}$). The contribution of denitrification to net N$_2$O emission varied across the land use types and ranged from 9 to 60%. Soil moisture was the key parameter regulating the partitioning of N$_2$O sources ($r^2 = 0.46$). Total N$_2$O fluxes were explained by a simple model ($r^2 = 0.83$) including parameters such as total dissolved nitrogen, organic carbon, and water content. A parsimonious model with the soil moisture content as a single scalar parameter explained 84% of methane flux variability across land uses. The assumption that 1% of the atmospherically deposited N on natural ecosystems is emitted as N$_2$O could be overestimated or underestimated (0.3–1.6%). The use of land-use-specific N$_2$O emission factors and further information on N$_2$O source partitioning should help constrain this uncertainty.

1. Introduction

Natural and seminatural terrestrial ecosystems (peatlands, forests, and grasslands) can play a significant role as nonagricultural sources or sinks of the main greenhouse gases (GHG), carbon dioxide (CO$_2$), methane (CH$_4$), and nitrous oxide (N$_2$O) (Dalal & Allen, 2008; Levy et al., 2012; Mander et al., 2010; Skiba et al., 2012). In the UK, 49% and 85% of the rural land cover in England and Wales, respectively, is composed of natural and seminatural land use types (Morton et al., 2011), which are currently collectively considered as net carbon sinks (land use, land use change, and forestry sector $-9$ Mt CO$_2$e in 2014; Brown et al., 2016). However, soil GHG emissions from nonfertilized native woodlands, mature poorly drained forests, and natural peatlands (not artificially drained or rewetted) are not currently reported to the UK GHG inventory (Brown et al., 2016). Indirect emissions due to atmospheric N deposition on natural ecosystems are not included in the national GHG inventory either, despite the uncertainty associated with the effect of increasing N deposition (Fowler et al., 2004, 2015) for N$_2$O emissions from natural forests (Skiba et al., 1998, 1999) and peatlands (Sgouridis & Ullah, 2015). For grasslands, the default Intergovernmental Panel on Climate Change (IPCC) Tier 1 emission factor approach (Intergovernmental Panel on Climate Change (IPCC), 2006), based on internationally agreed protocols using simple equations, is currently used, but this is not accurate enough to reflect regional variability driven by differences in land management intensity (e.g., intensive and extensive grasslands). To fill these gaps, there is an ongoing effort to develop and implement Tier 2 reporting systems that are country-specific taking into account climatic and management differences, for natural and seminatural ecosystems for a more accurate estimate of the annual GHG balance (Sgouridis & Ullah, 2015; Skiba et al., 2013). Further improvement and refinement of the Tier 2 reporting systems and the evolution of Tier 3 top-down modeling approaches can greatly benefit from linking landscape-scale flux measurements from natural and seminatural land use types with easily measurable environmental scalar parameters (Skiba et al., 2012, 2013).
Soil GHG fluxes, and particularly \( \text{N}_2\text{O} \) and \( \text{CH}_4 \), are compounded by large spatiotemporal variability with spikes of biogeochemical activity creating “hot spots” and “hot moments” (McClain et al., 2003). This high variability emanates from the environmental heterogeneity of the biotic (e.g., microbial consortia and vegetation) and abiotic (e.g., substrate availability, soil aeration/moisture, temperature, and pH) controls of the biogeochemical processes involved in the production or consumption of GHGs, and it is particularly difficult to simulate with process models that are applicable across natural ecosystem types (Dalal & Allen, 2008). Global methane emission controls include soil moisture or water table depth, soil carbon content, soil temperature and pH, and vegetation composition (Carter et al., 2012; Levy et al., 2012; Turetsky et al., 2014), but the relative importance of these controls varies depending on the spatial and temporal scales investigated (Dise, 1993; Levy et al., 2012) and also the ecosystem type. Soil temperature and moisture are also postulated as universal controls of respiratory \( \text{CO}_2 \) production across ecosystems (Gritsch, Zimmermann, & Zechmeister-Boltenstern, 2015).

Biological nitrous oxide production in soils is attributed to the aerobic processes of autotrophic and heterotrophic nitrification and nitrifier denitrification and the anaerobic processes of denitrification and dissimilatory nitrate reduction to ammonium (DNRA), with nitrification and denitrification being quantitatively the most important (Baggs, 2011; Butterbach-Bahl et al., 2013). Soil reactive N (labile organic bound N and inorganic \( \text{N} \) compounds) availability has been advocated as the major driver of \( \text{N}_2\text{O} \) emissions from soils (Butterbach-Bahl, 2006; Zhu et al., 2013). However, it was recently shown that low\( ^{15}\text{N} \) tracer application methods will proceed to completion with the reduction of \( \text{N}_2\text{O} \) to \( \text{N}_2 \) (Davidson et al., 2000). Recent methodological developments, reviewed in Baggs (2008) and Butterbach-Bahl et al. (2013), have allowed \( \text{N}_2\text{O} \) source partitioning to be applied under field conditions. While natural abundance stable isotope approaches (Pérez et al., 2006) are noninvasive and can provide valuable information on which processes may be involved in \( \text{N}_2\text{O} \) production, they are inconclusive on their own and cannot provide quantification information (Baggs, 2008). Stable isotope enrichment approaches that involve the addition of \( ^{15}\text{N}-\text{NH}_4^+ \) and/or \( ^{15}\text{N}-\text{NO}_3^- \) to soil have been the most reliable quantification method of \( \text{N}_2\text{O} \) source partitioning so far, but the need for large amounts of \( ^{15}\text{N} \) tracer application has restricted their use to fertilized agro-ecosystems (Baggs et al., 2003; Bateman & Baggs, 2005; Li et al., 2016; Mathieu et al., 2006) and forests receiving high rates of atmospheric N deposition (Ambus, Zechmeister-Boltenstern, & Butterbach-Bahl, 2006; Zhu et al., 2013). However, it was recently shown that low\( ^{15}\text{N} \) tracer application methods can be used in situ to quantify \( \text{N}_2 \) and \( \text{N}_2\text{O} \) fluxes from natural and seminatural ecosystems (Sgouridis, Stott, & Ullah, 2016; Sgouridis & Ullah, 2015) and this approach could be further expanded to account for \( \text{N}_2\text{O} \) source partitioning (Buchen et al., 2016; Morse & Bernhardt, 2013).

This paper reports in situ GHG fluxes from natural and seminatural ecosystems in two replicated UK catchments and elucidates the relative contribution of denitrification to net \( \text{N}_2\text{O} \) production. Our specific
objectives were to (1) assess the relative magnitude of GHG fluxes across a wide range of natural and seminatural land use types, (2) evaluate the environmental controlling factors of GHG fluxes within different land use types, (3) attempt to derive simple models for predicting GHG fluxes across ecosystem types, and (4) partition the \( \text{N}_2\text{O} \) flux between denitrification and other possible sources and assess the environmental controls affecting the source apportionment.

2. Methods

2.1. Study Sites

Two river catchments in the UK, the Conwy (area 345 km\(^2\); North Wales, 52°59'82"N, 3°46'06"W) and the Ribble-Wyre (area 1145 km\(^2\); NW England, 53°59'99"N, 2°41'79"W), were selected for this study. In both of these catchments more than 90% of land cover consists of natural and seminatural rural land use types (Morton et al., 2011). These catchments were chosen as priority catchments by the UK Natural Environment Research Council (NERC) Macronutrient Cycles Programme (www.ltls.org.uk) for investigating the magnitude and spatiotemporal variation of N, C, and P fluxes between soil, water, and air under a changing climate and perturbed C cycle.

In the Conwy catchment, four study sites (C-PB = peat bog; C-UG = unimproved grassland; C-IG = improved grassland; and C-MW = mixed woodland) were selected (Figure S1a in the supporting information). The headwater part of the Conwy River catchment lies at an average altitude of 440 m above sea level and has an average rainfall of 2200–2400 mm yr\(^{-1}\). The C-PB and C-UG are under a light grazing regime, less than one sheep per hectare, while the C-IG, characterized by seasonally waterlogged cambric stagnogley soils, is intensively grazed perennally by both sheep and cattle, while fertilizer (range: 100–200 kg N ha\(^{-1}\)) and manure are applied twice per year during spring and summer months (E. Ritchie, personal communication). The C-MW (mature mixed wood forest and currently unmanaged) is characterized by typical brown podzolic soils that are shallow and well drained, while bare rock is locally visible and steep slopes are common.

In the Ribble-Wyre catchment, four study sites (R-UG = unimproved grassland; R-IG = improved grassland; R-HL = heathland; and R-DW = deciduous woodland) were selected (Figure S1b in the supporting information). The dominant soils in the area have been described as stagnopodzols to stagnohumic gleys, the altitude ranges from 260 to 290 m above sea level, and the average rainfall is 1693 mm yr\(^{-1}\). The R-UG and R-IG have land management practices analogous to the ones described for the unimproved and improved grasslands in the Conwy catchment. Moreover, the R-UG was fertilized with N in the last decade once and has not been fertilized since, while it is being mowed twice per year (R. Rhodes, personal communication). The R-HL is privately owned, managed as a grouse moor, and grazed by sheep at low densities, while some recreational activities such as hiking are also allowed (Abbeystead Est., personal communication). The R-DW is an old growth forest developed on poorly drained soils and has never been fertilized (M. Colledge, personal communication). Further details on dominant plant species and soil texture of the study sites can be found in Sgouridis and Ullah (2014).

2.2. Gas Flux Measurements

Greenhouse gas fluxes were measured monthly using static chambers between April 2013 and October 2014 (total 17 months) with the exception of November 2013 and January 2014, which were not sampled. The measurement of greenhouse gas fluxes was part of a broader study aimed at quantifying in situ \( \text{N}_2 \) and \( \text{N}_2\text{O} \) fluxes due to denitrification from the same land use types (Sgouridis & Ullah, 2015) using an adapted \(^{15}\text{N}\) gas flux method (Mosier & Klemmedtsson, 1994) for low \(^{15}\text{N}\) tracer application, appropriate for natural and seminatural terrestrial ecosystems, which is described in detail in Sgouridis et al. (2016). Briefly, five plots were randomly established in each study site within each catchment amounting to 40 plots per monthly sampling campaign. In each plot a round PVC collar (basal area 0.05 m\(^2\); chamber volume 4 L) was inserted into the soil at ~10 cm depth (15 cm for the R-HL and C-PB plots) 2–4 weeks before the measurement date. The natural vegetation cover at the soil surface of each installed collar remained unchanged. The PVC collars were fitted with a circular groove of 25 mm depth to fit in an acrylic cylindrical cover (chamber) providing a gastight seal when filled with water, and the chamber was covered with reflective foil for minimizing temperature increase within the chamber headspace during the incubation period (Ullah & Moore, 2011). Labeled \(^{15}\text{NO}_3^-\) (98 at. % \(^{15}\text{N}\), Sigma-Aldrich) was applied in each plot at a mean rate between 0.03 (±SE 0.005)
and 0.50 (±SE 0.073) kg N ha$^{-1}$ via 10 injections of equal volume through a grid (4 × 6 cm) using custom-made 10 cm long lumber needles (15 cm for the R-HL and C-PB plots) attached to a plastic syringe (Rutting, Boeckx, et al., 2011). The tracer solution (50–200 mL) was adjusted between 3 and 5% of the ambient volumetric water content. For the natural land use types, the average tracer application rate reflected the current daily estimates of atmospheric N deposition in the UK (0.05 kg N ha$^{-1}$ d$^{-1}$) (Payne, 2014), while for the grassland soils the tracer application mimicked a daily fertilizer application rate of 0.5 kg N ha$^{-1}$ d$^{-1}$. The application of the $^{15}$N tracer at these low rates should not be expected to enrich the soil nitrate pool significantly and potentially affect greenhouse gas emissions, in excess of the amount of nitrogen normally deposited via natural processes and common management practices in these land use types (Sgouridis et al., 2016). Following the $^{15}$N tracer application, the collars were covered with the acrylic chamber fitted with a rubber septum for gas sampling. Two sets of gas samples (20 mL each) were collected with a gas tight syringe (SGE Analytical science) through the septum of the chamber cover at $T = 1$ h and $T = 2$ h, while a $T = 0$ h sample was collected immediately after tracer injection above the plot surface before fitting the chamber cover. The gas samples were transferred into pre-evacuated (<100 Pa) 12 mL borosilicate glass vials with butyl rubber septa (Exetainer vial; Labco Ltd., High Wycombe, United Kingdom) for storage under positive pressure and were analyzed within 8 weeks from collection without any significant change of the gas concentration (Laughlin & Stevens, 2003).

One set of the gas samples was analyzed for $^{15}$N-$N_2$ and $^{15}$N-$N_2O$ on an “in-house” laboratory-designed continuous flow isotope ratio mass spectrometer (CF-IRMS), and flux rates were calculated using the “non-equilibrium” equations as described in Sgouridis et al. (2016). The second set of gas samples was analyzed for total $N_2O$ ($^{14}$N+$^{15}$N-$N_2O$) on a GC-μECD and for $CH_4$ and $CO_2$ on a GC-FID (7890A GC Agilent Technologies Ltd., Cheshire, UK). The minimum detectable concentration difference (MDCD) was defined for each gas with repeated manual analyses of reference standards ($n = 8$, $N_2O = 0.3$ ppm, $CH_4 = 2$ ppm, and $CO_2 = 200$ ppm) and was calculated using the following equation (Matson, Pennock, & Bedard-Haughn, 2009):

$$\text{MDCD} = \mu_{\text{pair diff}} + (2\sigma_{\text{pair diff}})$$

where $\mu$ is the mean difference of all possible unique pairs of reference standards ($n = 28$) and $\sigma$ is the standard deviation between sample pairs. The MDCD values were 0.008, 0.036, and 3.6 ppm for $N_2O$, $CH_4$, and $CO_2$, respectively, while instrument precision was <1% relative standard deviation for all three gases. Only samples that were above or below (in case of consumption) the MDCD value for each gas were used for flux calculations, and if they were not, they were considered as zero fluxes. Flux rates were determined by linear regression of gas concentrations in the chamber headspace (adjusted for standard temperature and pressure) between 0 and 2 h, multiplied by the chamber volume and divided by the chamber area and time of incubation. The minimum detectable fluxes were 0.34 μg $N_2O$-N m$^{-2}$ h$^{-1}$, 0.68 μg $CH_4$-C m$^{-2}$ h$^{-1}$, and 67.14 μg $CO_2$-C m$^{-2}$ h$^{-1}$.

For estimating the ratio of denitrification-derived $N_2O$ over the total $N_2O$ emission (DN$N_2O$/TN$N_2O$), the DN$N_2O$ flux rates calculated from the CF-IRMS samples (Sgouridis & Ullah, 2015) were used. Where necessary, TN$N_2O$ flux rates were calculated based on 20 h incubation to correspond to the respective time interval of DN$N_2O$ fluxes. Sgouridis et al. (2016) have shown that due to the high spatial variability between plots within each land use type, mean DN$N_2O$ fluxes were not statistically different when estimated based on 1, 2, or 20 h incubation. Therefore, an assumed N$N_2O$ flux linearity during 20 h of incubation did not significantly affect flux rate estimation due to the high spatial variability of N$N_2O$ emissions, which is in line with the findings by Chadwick et al. (2014). When TN$N_2O$ fluxes were negative (indicating consumption), but the proportion of the denitrification-derived N$N_2O$ (DN$N_2O$) increased in the same sample, then all N$N_2O$ was assumed to be produced by denitrification. In case of zero TN$N_2O$ fluxes but detectable DN$N_2O$ flux, due to the lower limit of detection of the CF-IRMS (0.2 ng $N_2O$-N m$^{-2}$ h$^{-1}$), then all N$N_2O$ was assumed to be produced by denitrification. In the occasions where DN$N_2O$ flux was larger than the TN$N_2O$ flux (10% of total sample pool), then 100% of the N$N_2O$ flux was assumed to derive from denitrification. Finally, in 3% of the samples, DN$N_2O$ was not measured successfully due to sampling or analytical bias, and in these cases the ratio of DN$N_2O$/TN$N_2O$ was not estimated.

Annual greenhouse gas fluxes were estimated by interpolating monthly measurements for each year and calculating the average over the two monitoring years. The global warming potential (GWP) for each land use type was calculated for the 100 year horizon, including climate-carbon feedback, as CO$_2$ equivalent by
multiplying average annual N₂O and CH₄ fluxes with the conversion factors of 298 and 34, respectively (IPCC, 2013).

Five composite soil samples (0–10 cm) were collected with a hand auger from each study site after the end of each gas flux measurement within 50 cm of each plot. The samples were transported to the laboratory on ice and stored at 4°C overnight. The analytical procedures for determining key soil properties are detailed in Text S1 in the supporting information.

2.3. Statistical Analysis

Factor analysis was used in Sgouridis and Ullah (2015) to cluster individual sites into distinct land use types. The sites C-PB, C-UG, and R-HL formed a distinct group called organic soils (OS), the sites C-IG and R-IG clustered together, forming the improved grassland (IG) land use type, while the site R-UG formed a separate land use type, named as semi-improved grassland (SIG). Finally, the mixed woodland (MW) in the Conwy catchment and the deciduous woodland (DW) in the Ribble-Wyre catchment were also considered as separate natural land use types. All subsequent results and statistical tests refer to the above land use type groups. Three-way analysis of variance (ANOVA) was performed for comparing the variance of the means between land use types, seasons, and years. Multiple linear regression was used for exploring the factors controlling greenhouse gas fluxes within and across land use types. All statistical analyses were performed using SPSS® 23.0 for Windows (IBM Corp., 2015, Armonk, NY) and are described in Text S2 in the supporting information.

3. Results

3.1. Soil Properties

The organic soil (OS) land use type (peat-bog, acid grassland, and heathland) was characterized by high soil organic matter and moisture contents and low bulk density, soil nitrate, and pH (Table 1). Conversely, the improved grasslands (IG) had significantly higher bulk density, nitrate, and ammonium contents as well as labile carbon availability (as indicated by the higher CO₂ production rate). The mixed woodland (MW) in the Conwy catchment, even though unmanaged, had similar nitrate and ammonium contents to the improved grasslands, but significantly lower soil moisture and pH compared to the IG (Table 1). The site R-UG formed a distinct land use type, named as semi-improved grassland (SIG), with intermediate soil properties between OS and IG (Table 1). Finally, the deciduous woodland (DW) in the Ribble-Wyre catchment had significantly higher soil pH, while it displayed similar soil moisture and nitrate contents to the SIG.

Apart from the highly significant differences of the soil properties between the five land use types (Table S1 in the supporting information), certain soil properties displayed significant seasonal variation most notably the water-filled pore space (WFPS), soil nitrate content, and soil temperature shown in Table S2 in the supporting information. The seasonal variation pattern was similar between all land use types with decreasing WFPS from winter to summer and increasing soil nitrate in the same seasonal order, while the soil temperature displayed a typical temperate seasonal cycle. The three-way ANOVA indicated significant intra-annual variation for the soil moisture, nitrate, dissolved organic carbon (DOC), and total dissolved nitrogen (TDN) contents with the second year having lower moisture (drier soil conditions), nitrate and TDN, and higher DOC values (data not shown).

3.2. Nitrous Oxide Fluxes and the Ratio DN₂O/TN₂O

The mean total N₂O fluxes (from all possible sources) were significantly different among land use types (ANOVA; F = 171.4, d.f. = 4, p < 0.001) and ranged between −1.32 and 312.3 μg N m⁻² h⁻¹ (Figure 1a). Consumption of nitrous oxide (significant negative fluxes) was exclusively observed in the OS land use type, but on average OS was a minor source of N₂O about 40 times smaller than the IG, which emitted 3 times more N₂O than the SIG. The mean contribution of denitrification to the total N₂O flux (DN₂O/TN₂O) ranged between 0.09 and 0.59 and was lowest in the well-drained MW forest and highest in the poorly drained DW forest, while in the OS, IG, and SIG land uses was on average 0.44 (Figure 1a). The ratio DN₂O/TN₂O increased seasonally in the order spring < summer < autumn < winter (Figure 1c). There was no consistent seasonal pattern of total N₂O emissions across the different land use types (Figure 1b), while event-related emissions were observed in both the SIG and IG land uses (e.g., N₂O emissions peaked after fertilization
Table 1

| Soil Physico-chemical Properties in the Five Land Use Types in the Conwy and Ribble-Wyre River Catchments |
|---|
| **OS - organic soils (n = 255)** | **MW - mixed woodland (n = 85)** | **DW - deciduous woodland (n = 85)** | **SIG - semi-improved grassland (n = 85)** | **IG - improved grassland (n = 170)** |
| Bulk density (g cm$^{-3}$) | 0.09 (0.06)$^a$ | 0.56 (0.015)$^B$ | 0.38 (0.010)$^c$ | 0.29 (0.009)$^d$ | 0.59 (0.008)$^b$ |
| WFPS (%) | 69 (0.5)$^a$ | 39 (1.0)$^b$ | 65 (0.4)$^c$ | 67 (1.0)$^ac$ | 60 (0.8)$^d$ |
| Moisture content (% on w/w) | 86 (0.5)$^a$ | 38 (0.5)$^b$ | 60 (0.6)$^c$ | 64 (1.0)$^d$ | 42 (0.5)$^e$ |
| pH | 4.2 (0.03)$^a$ | 5.2 (0.08)$^b$ | 7.0 (0.04)$^c$ | 5.8 (0.03)$^d$ | 6.0 (0.01)$^a$ |
| Soil temperature (°C) | 10 (0.2)$^a$ | 10 (0.3)$^ab$ | 11 (0.3)$^b$ | 12 (0.4)$^bc$ | 12 (0.3)$^c$ |
| Organic matter (%) | 90 (0.9)$^a$ | 12 (0.3)$^b$ | 25 (0.5)$^c$ | 51 (1.4)$^d$ | 18 (0.4)$^e$ |
| DOC (g m$^{-2}$) | 6.0 (0.27)$^a$ | 4.3 (0.46)$^b$ | 4.5 (0.30)$^b$ | 10.5 (0.67)$^c$ | 4.3 (0.23)$^b$ |
| CO$_2$ production (mg C m$^{-2}$ h$^{-1}$) | 43.8 (1.99)$^a$ | 36.6 (2.00)$^a$ | 53.3 (3.45)$^b$ | 75.9 (4.87)$^c$ | 160.3 (5.96)$d$ |
| C:N (g g$^{-1}$) | 0.11 (0.012)$^a$ | 0.32 (0.030)$^b$ | 0.05 (0.007)$^c$ | 0.19 (0.075)$^a$ | 0.42 (0.053)$b$ |
| NO$_3$ -N (g m$^{-2}$) | 0.02 (0.002)$^a$ | 0.43 (0.046)$^b$ | 0.14 (0.010)$c$ | 0.17 (0.013)$^c$ | 0.38 (0.020)$b$ |
| NH$_4$ -N (g m$^{-2}$) | 0.37 (0.018)$^a$ | 0.74 (0.052)$^b$ | 0.41 (0.030)$c$ | 0.80 (0.059)$^b$ | 0.80 (0.034)$b$ |

Note. Data are mean ± standard error (SE) in parenthesis. Same lower case letters indicate no significant differences (p > 0.05) between land use types according to three-way ANOVA (one-way ANOVA for CN ratio) and the Hochberg’s GT2 post hoc test. For CN ratio, n = 30 for organic soils, n = 10 for mixed and deciduous woodland and semi-improved grassland, and n = 20 for improved grassland. WFPS, water-filled pore space; DOC, dissolved organic carbon; TDN; Total dissolved nitrogen.

and/or rainfall events). The drier second sampling year (2014) led to significantly lower N$_2$O emissions particularly in the SIG and IG, while the ratio DN$_2$O/TN$_2$O was lower only in DW (Table S3).

The multiple linear regression (MLR) analysis indicated that 37% of the variance in total N$_2$O fluxes could be explained by the availability of labile organic carbon (as expressed by the soil respiration rate) and nitrate, with an additional 6% explained by soil temperature and pH amounting to 43% (Table 2). Averaging fluxes per sampling plot (n = 40), thus removing the temporal but retaining the spatial variability, resulted in a simpler regression model with significantly increased predictive power (log$_{10}$(TN$_2$O + 0.03) = 0.1 + 0.001xTDN + 0.007xCO$_2$ – 0.018x volumetric water content (VWC); r$^2 = 0.85$).

The organic carbon availability was the key controlling factor of nitrous oxide emissions from the nitrate-rich soils of IG and MW, as well as the poorly drained DW forest soils. In the case of the organic carbon-rich soils of OS and SIG, higher N$_2$O emissions were associated with lower soil moisture and higher nitrate availability. The MLR analysis also showed that the ratio DN$_2$O/TN$_2$O is primarily controlled by the soil water content consistently across the different land use types, while secondary control factors include the soil pH, total dissolved nitrogen, and carbon availability (Table 2). In the poorly drained DW forest, showing the highest contribution of denitrification to N$_2$O emissions, the above soil parameters could explain 40% of the variance in the ratio DN$_2$O/TN$_2$O. Higher soil moisture content and pH also contributed to higher DN$_2$O/TN$_2$O ratio in the MW well-drained forest soils (explanatory power 26%). Aggregating fluxes per sampling plot increased the predictive power of soil moisture for the average ratio of DN$_2$O/TN$_2$O to 46%.

### 3.3. Methane Fluxes

The mean methane fluxes (Figure 2a) were significantly different between all the land use types (ANOVA; F = 205.9, d.f. = 4, p < 0.001) and ranged between −41.1 and 3474.5 μg CH$_4$-C m$^{-2}$ h$^{-1}$. The OS, as expected, were net sources of CH$_4$ throughout the year (Figure 2b) with highest fluxes observed during summer and autumn months. The poorly drained soils of DW in the Ribble-Wyre catchment were also a significant CH$_4$ source particularly during winter. While the IGs were minor sinks of atmospheric CH$_4$, the SIG (representative of extensive grassland management) was a minor net source throughout the year (Figure 2b). Finally, the well-drained soils of MW in the Conwy catchment were net sinks of atmospheric CH$_4$. There was no significant difference of methane fluxes between the two monitoring years for most land use types, with the exception of DW and SIG which showed lower CH$_4$ emissions in 2014 (Table S3).

The MLR analysis of monthly methane fluxes across all land use types (n = 680) with selected soil physicochemical variables (Table 2) showed that soil water content (measured as volumetric water content at 10 cm depth) can account for 59% of the variability in methane fluxes across natural and seminatural land use types, with an additional 3% explained by soil organic matter content and pH. MLR within each land...
Figure 1. (a) Average total N\textsubscript{2}O and the ratio DN\textsubscript{2}O/TN\textsubscript{2}O, (b) seasonal averages of total N\textsubscript{2}O, and (c) seasonal averages of DN\textsubscript{2}O/TN\textsubscript{2}O in the five land use types in the Conwy and Ribble-Wyre River catchments. Same lower case letters indicate no significant differences (p > 0.05) between land use types (a) or seasons within land use types (b and c) according to one-way ANOVA and the Games-Howell post hoc test. The sample size (n) is given in parenthesis for each land use type on the x axis in the order (spring, summer, autumn, and winter). The error bars indicate ±1SE.
Table 2

Multiple Linear Regression Analysis Results Between (a) Methane Fluxes, (b) Carbon Dioxide Fluxes, (c) Total N2O Fluxes, and (d) the Ratio DN2O/TN2O and Selected Soil Physico-chemical Properties

| Dependent variable | Equation | $r^2$ | n  |
|--------------------|----------|-------|----|
| (a) Methane         |          |       |    |
| All land use types  | $-1084.5 + (13.8xVWC) + (3.5xOM) + (55.2xPH)$ | 0.62  | 680 |
| Organic soils       | $-1311.2 + (146.8xPH) + (13.2xVWC) + (20.8xT)$ | 0.40  | 255 |
| Deciduous woodland  | $169.8 - (66.6xT) + (150.4xPH)$ | 0.60  | 85  |
| Semi-improved grassland | $-1615 + (18.2xVWC) + (124.1xPH)$ | 0.47  | 85  |
| Improved grassland  | $-40.4 - (25.2xT) + (4.9xWFPS)$ | 0.39  | 170 |
| (b) Carbon dioxide   |          |       |    |
| All land use types  | $-180.6 + (13.3xT) + (111.9xBD) + (1.2xWFPS)$ | 0.60  | 680 |
| Organic soils       | $-98.2 + (11.5xT) + (17xPH) - (0.7xWFPS)$ | 0.69  | 255 |
| Mixed woodland      | $-164.9 + (11.9xT) + (1.8xWFPS)$ | 0.58  | 85  |
| Deciduous woodland  | $-47.2 + (10.3xT)$ | 0.49  | 85  |
| Semi-improved grassland | $170.7 + (7.4xT) - (28.7xPH)$ | 0.40  | 85  |
| Improved grassland  | $-94.8 + (13.1xT) + (3.8xM)$ | 0.57  | 170 |
| (c) Total N2O       | $-25.7 + (0.17xCO2) + (0.03xNO3\textsuperscript{-}) + (5.1xPH) - (1.2xT)$ | 0.43  | 629 |
| Organic soils       | $57 - (0.45xM) - (8.2xPH) - (0.1xNO3\textsuperscript{-})$ | 0.20  | 228 |
| Mixed woodland      | $-21.8 - (0.41xCO2) + (2xT)$ | 0.46  | 79  |
| Deciduous woodland  | $-67 + (0.18xCO2)$ | 0.21  | 80  |
| Semi-improved grassland | $80.5 - (0.72xWFPS) + (0.07xNO3\textsuperscript{-}) - (0.01xTDN) - (0.28xOM)$ | 0.42  | 81  |
| Improved grassland  | $-123.3 + (0.13xCO2) - (1.9xT) + (26.6xPH)$ | 0.23  | 161 |
| (d) DN2O/TN2O       |          |       |    |
| All land use types  | $-0.88 + (0.02xWFPS) + (0.16xPH) + (0.001xTDN) + (0.002xCO2)$ | 0.22  | 606 |
| Mixed woodland      | $-2.4 + (0.35xPH) + (0.03xVWC)$ | 0.26  | 67  |
| Deciduous woodland  | $-1.03 + (0.05xVWC) - (0.004xCO2) + (0.001xTDN)$ | 0.40  | 80  |
| Semi-improved grassland | $-0.55 + (0.04xVWC)$ | 0.23  | 81  |
| Improved grassland  | $1.007 + (0.001xNO3\textsuperscript{-}) + (0.001xNH4\textsuperscript{+}) + (0.01xVWC)$ | 0.17  | 160 |

Note: The GHG flux data sets (a, b, and c) were normalized using the two-step approach, while the ratio DN2O/TN2O (d) was log10-transformed before the regression (for details see Text S2 in the supporting information). All regressions are significant at $p < 0.01$. VWC, volumetric water content; OM, organic matter content; T, soil temperature; WFPS, water-filled pore space; BD, bulk density; M, soil moisture; NO3, nitrate content; CO2, soil respiration; TDN, total dissolved nitrogen content; NH4\textsuperscript{+}, ammonia content.

use type revealed some further land use type-specific relationships (Table 2). Soil pH appeared to be an important controlling factor of methane fluxes in organic soils (21% explanatory power), with increased emissions associated with higher soil pH, while soil water content and temperature combined explained an additional 19% of methane flux variability in OS. Soil temperature alone explained 36% of the methane flux variability in DW and IG respectively, by indirectly affecting soil water availability during summer months (Figure 2b). Finally, in SIG the soil water content and pH combined accounted for 47% of the variability in methane fluxes. When the monthly fluxes were averaged per plot ($n = 40$) the soil water content alone explained 84% of the methane flux variation ($\log_{10}(\text{CH}_4 + 20) = -0.936 + 0.05x\text{VWC}$; $r^2 = 0.84$).

### 3.4. Carbon Dioxide Fluxes

The rate of CO2 production (including soil microbial respiration, roots, and that of any aboveground vegetation enclosed by the static chamber) differed significantly between natural and seminatural land use types (ANOVA; $F = 117.8$, d.f. = 4, $p < 0.001$) and ranged between 0.63 and 435.5 mg C m$^{-2}$ h$^{-1}$. The IG emitted 4 times more carbon dioxide than the OS and MW land uses and twice as much as the SIG (extensively managed) (Figure S2a in the supporting information). The seasonal variability was consistent across all the land use types with larger CO2 emissions observed in the summer and lowest in winter months (Figure S2b in the supporting information), while there were slightly higher emissions in the second drier year. Consequently, the soil temperature was shown to be the main controlling factor of CO2 emissions across land uses ($\log_{10}(\text{CO}_2) = -1.053 + 0.26xT; r^2 = 0.86$). The soil temperature was consistently the most important controlling factor highlighted by MLR analysis within each land use type (Table 2), while the soil moisture was also a significant regulator in the drier IG and MW sites.
3.5. Annual Fluxes and Global Warming Potential

The average annual CH$_4$ flux across the natural and seminatural land use types ranged between $-0.9$ and $18.7$ kg C ha$^{-1}$ yr$^{-1}$, while the respective annual N$_2$O flux range was $0.05$–$1.98$ kg N ha$^{-1}$ yr$^{-1}$. The greenhouse gas budget for each land use type, calculated using the 100 year horizon global warming potential (GWP), was dominated by the soil and ground vegetation respiration of carbon dioxide (Table 3), while the net ecosystem exchange of CO$_2$ was not measured in this study. The contribution of both methane and nitrous oxide to the GWP was highest in the IG, almost entirely due to N$_2$O emission, followed by the OS, where CH$_4$ emission contributed 97.5% of the GWP. Methane was also the main contributor (94%) to GWP from the poorly drained forest soils of DW, whereas methane consumption in the well-drained forest soils of MW reduced the GWP by 49%. In the extensively managed grassland (SIG) both greenhouse gases contributed considerably to the GWP, which was almost 3 times lower than the GWP of the improved grasslands.
Table 3
Annual Average Greenhouse Gas Fluxes, Total Global Warming Potential (GWPtotal), and the Contribution of Methane and Nitrous Oxide to the Global Warming Potential (GWP\textsubscript{N2O} + CH\textsubscript{4}) in the Five Land Use Types in the Conwy and Ribble-Wyre River Catchments

| Land use types     | N\textsubscript{2}O (kg N ha\textsuperscript{-1} yr\textsuperscript{-1}) | CH\textsubscript{4} (kg C ha\textsuperscript{-1} yr\textsuperscript{-1}) | CO\textsubscript{2} (kg CO\textsubscript{2eq} ha\textsuperscript{-1} yr\textsuperscript{-1}) | GWP\textsubscript{total} (kg CO\textsubscript{2eq} ha\textsuperscript{-1} yr\textsuperscript{-1}) | GWP\textsubscript{N2O} + CH\textsubscript{4} (kg CO\textsubscript{2eq} ha\textsuperscript{-1} yr\textsuperscript{-1}) | N\textsubscript{2}O (kg N ha\textsuperscript{-1} yr\textsuperscript{-1}) | CH\textsubscript{4} (kg C ha\textsuperscript{-1} yr\textsuperscript{-1}) |
|--------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Organic soils      | 22.1                     | 849.9                    | 11044.2                  | 11916                    | 872                      | 0.05                     | 18.7                     |
| Mixed woodland     | 123.5                    | –40.6                    | 9419.4                   | 9502                     | 83                       | 0.26                     | –0.9                     |
| Deciduous woodland | 48.2                     | 733.1                    | 13677.6                  | 14459                    | 781                      | 0.10                     | 16.2                     |
| Semi-improved grassland | 302.2               | 51.6                     | 18988.7                  | 19343                    | 354                      | 0.65                     | 1.1                      |
| Improved grassland | 928.9                    | –0.2                     | 39531.0                  | 40460                    | 929                      | 1.98                     | 0.0                      |

4. Discussion

4.1. Nitrous Oxide Fluxes and the Ratio DN\textsubscript{2}O/TN\textsubscript{2}O

The application of \textsuperscript{15}N tracer for measuring in situ the flux of N\textsubscript{2} and N\textsubscript{2}O due to denitrification (Sgouridis et al., 2016; Sgouridis & Ullah, 2015) also enabled the estimation of the ratio of denitrification-derived N\textsubscript{2}O over the total N\textsubscript{2}O emission (DN\textsubscript{2}O/TN\textsubscript{2}O) under field conditions and for the first time across a wide spatial and temporal range in natural terrestrial ecosystems. The \textsuperscript{15}N-N\textsubscript{2}O emitted from a uniformly labeled soil NO\textsubscript{3}\textsuperscript{–} pool can be attributed to the process of denitrification (DN\textsubscript{2}O) when assuming negligible dissipatory nitrate reduction to ammonium (DNRA) and immobilization and remineralization of \textsuperscript{15}N-NO\textsubscript{3}\textsuperscript{–} (Buchen et al., 2016; Zhu et al., 2013). Currently, there is no available methodology to discriminate N\textsubscript{2}O emissions between denitrification and DNRA (Baggs, 2008, 2011), and for this study we have assumed negligible contribution from DNRA, which when quantitatively important in temperate ecosystems can constitute up to 10% of the denitrification activity (Rutting, Huygens, et al., 2011; Sgouridis et al., 2011). The unlabeled N\textsubscript{2}O flux could be due to the contributions from autotrophic or heterotrophic nitrification and/or nitrifier denitrification (Buchen et al., 2016; Zhu et al., 2013), and further discrimination between these sources would have required double tracer labeling (Baggs et al., 2003; Bateman & Baggs, 2005; Mathieu et al., 2006; Morse & Bernhardt, 2013) and/or the use of natural abundance isotopomer approaches (Mander et al., 2014; Schmidt et al., 2004; Sutka et al., 2006) which, although desirable, was beyond the scope of this study.

The highest (mean 0.59) contribution of denitrification to the emission of N\textsubscript{2}O was observed in the poorly drained DW forest, while the lowest (mean 0.09) was measured in the well-drained MW forest (Figure 1a). Soil moisture has been shown as the key factor regulating the partitioning of N\textsubscript{2}O between denitrification and nitrification sources (Baggs et al., 2010; Bateman & Baggs, 2005; Li et al., 2016; Mathieu et al., 2006; Morse & Bernhardt, 2013), and this was confirmed by the MLR analysis in all the land use types (Table 2). In the case of the MW forest, which displayed the lowest WFPS and the highest availability of mineral N (Table 1), there was strong indication that nitrification rather than denitrification may be quantitatively more important as an N\textsubscript{2}O source (Ambus et al., 2006; Matson et al., 2009; Ullah & Moore, 2011). This is also supported by the highest nitrification potential of the MW shown in a preliminary study (Sgouridis & Ullah, 2014). This suggests that enhanced nitrification due to increased N inputs in well-drained forest soils could consequently lead to increased N\textsubscript{2}O emissions (Ambus et al., 2006). Conversely, the dominant N\textsubscript{2}O source in the hydromorphic forest soils (mean WFPS 65%) of the DW was denitrification (Ullah & Moore, 2011), while nitrification activity may have also been limited by the lowest ammonium availability (Table 1). In the grasslands (IG and SIG), the average WFPS range between 60 and 67% led to higher N\textsubscript{2}O emissions from nitrification sources (0.54–0.64), while the ratio of DN\textsubscript{2}O/TN\textsubscript{2}O increased with increasing soil moisture (Figure 1c and Table 2), which is in line with previous findings from laboratory incubation studies in grasslands (Bateman & Baggs, 2005; Mathieu et al., 2006; Stevens et al., 1997). Intermediate soil moistures (around 65% WFPS in grasslands) create optimal conditions for the simultaneous occurrence of nitrification and denitrification (Davidson et al., 2000) due to the uninhibited diffusion of both substrates and O\textsubscript{2} (Parton et al., 1996) and the occurrence of anaerobic microsites in soil aggregates (Sexstone et al., 1985). Our study highlights the quantitative importance of both nitrification and denitrification as N\textsubscript{2}O sources under field conditions and also shows that their relative magnitude varies seasonally (Wolf & Brumme, 2002) driven by seasonal changes in soil moisture, which should be taken into account in mitigation strategies for reducing N\textsubscript{2}O emissions from grasslands. Despite the high WFPS of the organic soils all year round (mean 69%), only 0.48 of the N\textsubscript{2}O...
emissions was attributed to denitrification with the ratio of DN2O/TN2O rising to 0.54 and 0.52 in summer and autumn, respectively (Figure 1c). At 70% WFPS it has been shown that all of the N2O emitted from grassland soils originated from denitrification (Bateman & Baggs, 2005), while at pH < 4.5 denitrification is quantitatively more important than nitrification (Baggs et al., 2010; Cheng et al., 2015). It is possible that nonhomogeneous distribution of the 15N-NO3 tracer and potential gas loss through subsoil gas diffusion or further reduction of 15N-N2O to 15N-N2 (Sgouridis et al., 2016) in the saturated highly porous peat soils may have led to underestimated contribution of denitrification to N2O emissions from the OS. There is currently a lack of studies on source partitioning of N2O emissions from natural peat/organic soils, with the exception of Pihlatie et al. (2004) where the unreliable acetylene inhibition technique (Baggs, 2008) was used for source partitioning. Our study is the first one to indicate that sources other than denitrification may contribute to N2O emissions from this under-represented soil type. However, further investigation is warranted to elucidate the various potential pathways of N2O emission using multiple tracer and/or natural abundance approaches.

Total nitrous oxide fluxes were highest from the improved grasslands, which emitted 3 times as much N2O as the SIG extensively managed grassland (Imer et al., 2013; Skiba et al., 2013). The mean annual nitrous oxide flux rate from the improved grasslands (2 kg N2O-N ha−1) corresponds to the lower range of the fluxes reported for similarly managed grasslands (N fertilization range: 150–200 kg N ha−1 yr−1) in the UK and Ireland (Cardenas et al., 2010; Rafique, Hennessy, & Kiely, 2011; Renou-Wilson et al., 2014; Skiba et al., 2013). The N2O fluxes from the mixed and deciduous forest soils in this study (0.1–0.3 kg N2O-N ha−1 yr−1) were comparable to nitrous oxide fluxes from temperate and boreal forests (deciduous and coniferous) in Canada (Matson et al., 2009; Peichl et al., 2010; Ullah & Moore, 2011) under moderate atmospheric N deposition (<20 kg N ha−1 yr−1), while they corresponded to the lower end of the fluxes reported for coniferous and deciduous European forests (Eickenscheidt et al., 2014; Luo et al., 2012; Pilegaard et al., 2006) at higher atmospheric N deposition (20–35 kg N ha−1 yr−1). The mean annual N2O efflux from the organic soils (0.05 kg N2O-N ha−1 yr−1) was significantly lower than from any other land use type and comparable only to effluxes from natural, pristine European peatlands (Carter et al., 2012; Leppelt et al., 2014), while it was 1–2 orders of magnitude lower than nitrous oxide emissions from organic soils used for agriculture (Petersen et al., 2012) or from drained peatlands (Mander et al., 2010) or peat extraction sites (Leppelt et al., 2014).

The multiple linear regression analysis indicated significant and relatively complex environmental controls of nitrous oxide emission from natural terrestrial ecosystems (Table 2) including nitrate and organic carbon availability, soil pH, and temperature with overall lower predictive power (43%) compared to the models predicting methane and carbon dioxide effluxes from the same land use types. Weak relationships between N2O fluxes and environmental variables measured in the field are commonly found in the literature (Carter et al., 2012; Eickenscheidt et al., 2014; Luo et al., 2012; Skiba et al., 2013). This has been attributed to the highly dynamic nature of nitrous oxide production and consumption processes and their enormous spatiotemporal variability (Baggs, 2011; Butterbach-Bahl et al., 2013), which is difficult to predict particularly at weekly or monthly timescales (Luo et al., 2012). Aggregating fluxes per sampling plot resulted in a simpler regression model that encompasses the key environmental drivers of N availability in the form of total dissolved nitrogen concentration in soil water (69% predictive power), carbon availability as expressed by the soil respiration rate (12% predictive power), and soil moisture as VWC (4% predictive power) and is applicable across different natural terrestrial ecosystem types.

Soil reactive N (labile organic bound N and inorganic N compounds) availability has been advocated as the major driver of N2O emissions from soils (Butterbach-Bahl et al., 2013), and the highly significant correlation \( r^2 = 0.69 \) (Figure 3a) between total N2O fluxes and TDN highlights that not only nitrate (the substrate of denitrification) but also ammonium (the substrate of nitrification) and organic N forms (supply of nitrate and ammonium through mineralization) exert significant control over the emission of N2O from multiple microbial processes. Sgouridis and Ullah (2014, 2015) have already shown that a gradient of N and organic carbon availability across the same natural and seminatural land use types, as this is influenced by management practices (e.g., fertilization, grazing, and hay cutting) in grasslands and natural variability in forests and organic soil, are the most significant proximal controls of denitrification activity and the associated N2O emissions. Fertilization and grazing with mineral nitrogen in the improved grasslands are the primary reasons for the higher N2O emissions from this land use type, and this dose-response relationship has been well documented in the literature (Cardenas et al., 2010; Rafique et al., 2011; Skiba et al., 2013) and likely explains
the 3 times lower emissions from the unfertilized SIG. Conversely, the N₂O emission from the OS was limited by nitrate supply as indicated by the MLR (Table 2) and also by the significantly lower nitrification and denitrification potentials compared to the other land use types (Sgouridis & Ullah, 2014).

4.2. Methane Fluxes

Land use type significantly affected methane fluxes, with the order of CH₄ emission rates being organic soils > poorly drained forest > semi-improved grassland > improved grassland > well-drained forest, which is in line with previous studies in rural ecosystems (Czóbel et al., 2010; Mander et al., 2010). The OSs (including an ombrotrophic peat bog, an acid grassland, and a heathland) were significant CH₄ sources at an average annual rate of 25 kg CH₄ ha⁻¹, which corresponds to the lower range reported for organic soils in the UK (Levy et al., 2012), while it is lower than the mean flux from temperate pristine wetlands across the globe (Turetsky et al., 2014) and in Estonia (Mander et al., 2010). Poorly drained forest soils were the second most important CH₄ source (annual mean 21.6 kg CH₄ ha⁻¹) at similar magnitude as deciduous forests on hydromorphic soils in the St. Lawrence River catchment, eastern Canada (Ullah & Moore, 2011), and in Estonia (Mander et al., 2010). Conversely, the well-drained soils of the mixed forest in the Conwy catchment were a small methane sink (annual mean 1.2 kg CH₄ ha⁻¹) comparable to methane uptake rates reported for automorphic forest soils in Europe (Luo et al., 2012; Mander et al., 2010) and Canada (Ullah & Moore, 2011). The improved grassland was on an annual basis methane neutral, while the semi-improved grassland was a small methane source (annual mean 1.5 kg CH₄ ha⁻¹), results that generally agree with previous studies of the GHG balance of grasslands in similar ecoregions (Imer et al., 2013; Petersen et al., 2012; Renou-Wilson et al., 2014; Skiba et al., 2013).

The multiple linear regression analysis on averaged plots over time highlighted the soil water content as the key environmental driver (84% explanatory power) of methane flux across natural and seminatural land use types (Figure 3b). Previous studies have shown that averaging measurements over time can lead to stronger relationships between CH₄ flux and environmental variables as random measurement errors are canceled out (Levy et al., 2012), while temporal lags and “memory effects” in methanogenic activity are integrated (Turetsky et al., 2014). Soil water content is commonly used as a surrogate variable for soil O₂ concentration and is often a better proxy than water table depth for gauging the extent of methanogenesis (Imer et al., 2013; Levy et al., 2012). Significant methane emissions were measured at soil water contents between 55 and 70% VWC (OS and DW sites), while methane consumption was more prominent at <40% VWC (MW site), which is in agreement with previous investigations of the relationship between methane emissions and soil moisture (Schaufler et al., 2010). Soil organic matter content explained an additional 2% of the variation in methane fluxes across the land use types. Soil carbon availability has been shown to positively affect CH₄ emissions (Carter et al., 2012; Levy et al., 2012) directly through the supply of carbon substrate for methanogenesis, but also indirectly by increasing the water holding capacity of the soil (Hudson, 1994), thus leading to increased soil moisture content that hinders the aerobic decomposition of the available carbon (Jungkunst & Fiedler,
A significant positive relationship was found between methane emission and soil pH in the OS, DW, and SIG sites (main methane sources among the investigated land use types), which for the organic soils in particular explained 21% of the methane flux variation. Increase in pH even by 0.5 (from 3.5 to 4.0) in peatlands and shrublands has been associated with increased CH₄ efflux by almost 300% (Carter et al., 2012), which was attributed to increased DOC availability due to the increased solubility of organic acids and humic substances in the soil brought about by the higher pH (Evans et al., 2008; Murakami, Furukawa, & Inubushi, 2005). Overall, our findings highlight the importance of soil water content as a broad spatial scale environmental driver of methane emissions from terrestrial ecosystems, which is easily measurable and can be used as a scalar indicator for predicting methane emissions at the catchment scale. Further testing of this simple model across land use types in several catchments is warranted for assessing its applicability for the development of Tier 3 emission factor models.

4.3. Carbon Dioxide Fluxes

The carbon dioxide fluxes from our static chamber measurements represent the combined flux from heterotrophic soil respiration and autotrophic respiration of the vegetation enclosed by the chamber, which constitute a significant proportion of the ecosystem exchange of CO₂ (Parkin et al., 2005). The CO₂ fluxes between land use types followed the order grasslands > forests > organic soils, and the mean annual range (2.5–11 t CO₂-C ha⁻¹) is within the ranges reported for similarly managed grasslands in the UK (Skiba et al., 2013), European peatlands and shrublands (Carter et al., 2012), and temperate forest soils in Canada (Ullah & Moore, 2011), while being lower than temperate forest soils in Europe (Luo et al., 2012). The addition of low amounts of ¹⁵N tracer in the measured plots did not affect soil respiration rates as shown by Sgouridis et al. (2016), which is in agreement with previous studies on the effect of N fertilization on soil respiration (Carter et al., 2012; Liu & Greaver, 2009; Luo et al., 2012; Raich & Schlesinger, 1992; Skiba et al., 2013).

Soil temperature explained most of the variability (47%) of soil respiration across land use types, with an additional 13% explained by soil moisture and bulk density, which confirms the findings of several previous studies on the controls of soil CO₂ respiration in terrestrial ecosystems (Carter et al., 2012; Gritsch et al., 2015; Imer et al., 2013; Luo et al., 2012; Schaufler et al., 2010; Skiba et al., 2013). The temperature dependence of soil respiration can be explained by the stimulation of biological activity (plant roots and microbial communities) with increasing temperature, and this was evident within all the investigated land use types as indicated by the MLR analysis (Table 2). Temperature sensitivity is larger for relatively wet soils (WFPS: 60–70%) (Gritsch et al., 2015), which was shown for the OS and DW soils where soil temperature alone explained 67% and 49% of the CO₂ efflux variation. At very dry or very wet conditions soil moisture sensitivity over soil respiration also becomes important (Gritsch et al., 2015) and can limit CO₂ efflux either by restricting aeration and O₂ supply for aerobic respiration (wet conditions) or through osmotic stress of the microbial community under dry conditions (Smith et al., 2003). This could explain the observed negative relationship between soil respiration and WFPS in the OS sites (mean WFPS 70%) and the positive relationship between soil water content and CO₂ efflux in the MW and IG sites (mean WFPS 40–60%). When averaging measurements per sampling plot, soil temperature became the single scalar predictor (Figure 3c) of soil respiration across broad spatial scales within the soil water content range 40–70% WFPS, which is common across terrestrial ecosystems in northern latitudes.

4.4. Global Warming Potential and Emission Factors

The greenhouse gas (GHG) budget was estimated using the 100 year global warming potential, including climate-carbon feedback (IPCC, 2013), and for each land use type it was dominated by the CO₂ production (microbial plus root respiration, per se ecosystem respiration) representing more than 93% of the total GWP (Table 3). Of this ecosystem respiration term, 35–45% could be attributed to vegetation respiration (Silvola et al., 1996), particularly during the growth season between April and September, since plants were not removed from the collar enclosures. It has been shown that in similar land use types under comparable management regimes and climatic conditions (Petersen et al., 2012; Renou-Wilson et al., 2014; Skiba et al., 2013), the net ecosystem exchange of CO₂ is the dominant term of the GHG budget, with forests considered as annual net C sinks (Ullah & Moore, 2011), while in grasslands and organic soils the counteraction of the net ecosystem CO₂ exchange (NEE) by the emission of N₂O and CH₄, respectively, varies spatiotemporally due to climatic and management effects. In this study we were not able to estimate the NEE or fluvial carbon
were able to estimate the relative contribution of N$_2$O and CH$_4$ emission to GWP and have observed a similar agriculture-related indirect N$_2$O emissions calculated by the IPCC Tier 1 methodology. The assumption that further lead to increased GWP contributions by both N$_2$O and CH$_4$ (Petersen et al., 2012). Poorly drained forests were the third highest GWP contributor (5% CH$_4$ and 0.3% N$_2$O), mainly due to methane emissions, and this should be taken into account in forest restoration strategies for C sequestration where an increased proportion of wetland forest soils has been suggested to optimize C sequestration (Ullah & Moore, 2011). Conversely, in well-drained forest soil, methane consumption was counteracted by a 33% contribution of N$_2$O emission to GWP reducing the source strength of GWP by an order of magnitude compared to the other land use types. Nitrous oxide emission factors calculated as a fraction of mineral and/or organic N inputs (fertilizer and manure, IG or manure application only, SIG) averaged at 0.4 and 0.9% for the semi-improved and improved grasslands respectively, which is at the lower end of the range of the default Tier 1 emission factor (0.3–3%; IPCC, 2006) and comparable to the emission factors estimated for fertilized seminatural grasslands in France (Klumpp et al., 2011).

Using simulated atmospheric N-deposition data for the UK (CEH Edinburgh, personal communication), we have estimated the fraction of N deposition-induced N$_2$O emissions from poorly drained forest (0.5%), well-drained forest (1.6%), and organic soils (0.3%) which corresponded to the lower range (0.2–5%) of the agriculture-related indirect N$_2$O emissions calculated by the IPCC Tier 1 methodology. The assumption that 1% of the deposited N on natural ecosystems is emitted as N$_2$O (IPCC, 2006) may overestimate this source in the case of organic soils or underestimate it in the case of N-rich well-drained forest soils, and the development of Tier 2 emission factors for natural and seminatural ecosystems, that take into account soil environmental conditions such as N and C availability and pH, is needed for more accurate prediction of N$_2$O emissions (Skiba et al., 2012).

5. Conclusion

The application of $^{15}$N tracer allowed us to estimate the relative contribution of denitrification to the total N$_2$O emission under field conditions and for the first time across a wide spatial and temporal range in natural terrestrial ecosystems. Up to 60% of N$_2$O emission was attributed to denitrification, while the quantitative importance of both nitrification and denitrification as N$_2$O sources varied across land use types primarily driven by differences in soil moisture content. This finding can prove useful when designing mitigation strategies for the reduction of N$_2$O emission, particularly in grasslands, while further studies are needed to elucidate the quantitative importance of the various N$_2$O sources in soils under natural ecosystems (peatlands and forests) especially under increased atmospheric N deposition and drainage scenarios. A gradient of N and organic carbon availability across the natural and seminatural land use types, as this is influenced by management practices (e.g., fertilization, grazing, and hay cutting) in grasslands and natural variability in forests and organic soils, was the most significant proximal control of N$_2$O emission. The landscape heterogeneity of methane flux was primarily explained by the soil water content, while additional environmental controls such as organic carbon content, soil temperature, and pH can be used to constrain the variability within ecosystem types and should be considered when deriving Tier 2 and 3 methane emission factors. Methane emissions from organic soils and N$_2$O emissions from improved grasslands contributed equally to the GWP, indicating that not only managed but also natural and seminatural ecosystems should be considered in climate change mitigation strategies. For nitrous oxide emission factors in particular, the assumption that 1% of the atmospherically deposited N on natural ecosystems is emitted as N$_2$O may be overestimated in the case of natural organic soils or underestimated in the case of N-rich well-drained forest soils, and additional measurements in these natural land use types are needed to further constrain this uncertainty.
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