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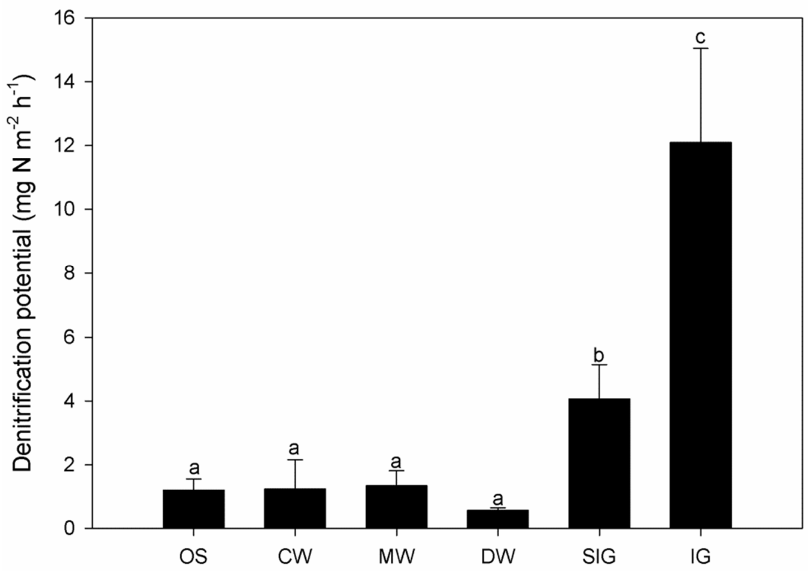
Figure for the Table of Contents Entry

Denitrification potential was significantly influenced by land use type where it was lower in organic and forests than in semi-improved and improved grassland soils.



Environmental Impact Statement

This paper investigated the denitrification potential (DP) and its biogeochemical controls. It was hypothesised that the relative magnitude of denitrification activity may be regulated, among others, by a gradient of soil nitrate (low to high) between organic (peat bog, heathland, and acid grassland), forest (coniferous and deciduous), and grassland (improved and semi-improved) rural land use types. Organic and forest soils had the lowest and semi-improved and improved grassland soils had the highest DP. Differences in soil nitrate concentration, availability of organic carbon, soil pH and texture between organic, forest and grassland soils as influence by land management practices and natural variability of the N cycle processes affected the observed DP. Based on the results, the paper discusses the importance of land use types in affecting the relative magnitude of denitrification activity and recommends its consideration when modelling the response of denitrification to land use change.

Denitrification potential of organic, forest and grassland soils in the Ribble-Wyre and Conwy River catchments, UK

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ABSTRACT

Soil denitrification activity can be highly variable due to the effects of varied land use management practices within catchments on the biogeochemical regulators of denitrification. To test this assumption in the context of mixed-use rural catchments, it was hypothesised that the relative magnitude of denitrification activity may be regulated, among others, by a gradient of soil nitrate (low to high) between organic (peat bog, heathland, and acid grassland), forest (coniferous and deciduous), and grassland (improved and semi-improved) rural land use types. The denitrification potential (DP) of organic, forest and grassland soils, in two UK catchments was measured in the laboratory. Land use type significantly ($p < 0.05$) influenced the DP, which ranged between 0.02 and 63.3 mg N m^{-2} h^{-1}. The averaged DP of organic and forest soils (1.08 mg N m^{-2} h^{-1}) was 3 and 10 times less than the DP of semi-improved (4.06 mg N m^{-2} h^{-1}) and improved (12.09 mg N m^{-2} h^{-1}) grassland soils, respectively; and among others, nitrate correlated positively ($p < 0.05$) with the DP. The results indicated that the difference in soil nitrate concentration between organic (naturally low in nitrate availability) and grassland soils (nitrate enriched due to land management) partially regulated the extent of DP. In the absence of N fertilisation, except for the atmospheric N deposition, the relatively low net nitrification potential (as a source of nitrate for denitrifier) of organic and forest soils alone seem to have resulted in lower denitrifier's activity compared to grassland soils. Moreover, the interactions between soil organic carbon, pH, bulk density, water filled pore space, and texture, as these are influenced by the relative degree of land management, exerted additional controls on the DP. The results suggest that land management can have significant effects on denitrification, and thus needs to be considered when modelling and/or predicting the response of denitrification to land use change.

1. Introduction

Biological denitrification is the reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to nitrous oxide (N_2O) and dinitrogen (N_2) gases.[1] Denitrification is important as a permanent removal mechanism of reactive nitrogen (oxidised and reduced forms) produced either *in situ* or as external inputs to soils through fertilisation and atmospheric nitrogen (N) deposition.[2] The relative magnitude of N_2O and N_2 gas production by denitrification in soils has implications for primary productivity, water quality and global warming.[2] However, soil denitrification is highly variable spatio-temporally within catchments with a multitude of land uses but also at the microsite scale due to the long and short term effects of land use management on the proximal biogeochemical regulators of denitrification such as the amount of nitrate, availability of organic carbon (C), and the concentration of soil oxygen (O_2).[3-5] Land management practices (e.g. cultivation, fertilisation, livestock grazing, and change of plant species cover) affect both the soil environmental conditions[6, 7] and the composition and diversity of denitrifying microbial populations.[8] To better understand how denitrification is regulated across different land use types and to be able to predict changes in denitrification in response to land use change, the controlling factors of denitrification need evaluation in the context of land use management.

Natural and semi-natural terrestrial ecosystems in the UK (i.e. peatlands, heathlands, acid grasslands, deciduous and coniferous forests), where there is no fertiliser use and the impact from grazing and forestry is minimal,[9] along with improved grasslands (fertilised and grazed intensively) constitute approximately 49 % and 85 % of rural land use cover in England and Wales, respectively.[10] Unlike arable agriculture, these land use types have been poorly investigated for their role in reactive N loss through denitrification, in contrast to N-mineralization studies.[11] Moreover, most of the available data on reactive N loss from soils generated in the last two decades in the UK have been on N_2O emissions within the context of N fertilisation of cultivated soils and the Kyoto Protocol.[12] Thus, there is a need to better understand factors that control net reactive N loss through denitrification in these rural land use types.

Denitrification potential (DP) can be used as a proxy for the concentration of denitrifying enzymes in a soil sample,[13] which in turn is determined by the environmental conditions to which denitrifier populations were exposed in the field at the time of soil sampling.[14] Therefore, DP is appropriate for comparing denitrifier activity between contrasting land use types,[15] for evaluating the controlling factors of the process,[16] and for assessing the effect of land use management on the soil environmental conditions and consequently denitrification activity.[7] While acknowledging the small scale and short term inherent variability of denitrification often creating 'hot moment' activity within 'hotspots',[17] a replicated approach (both in space and time) in measuring DP can provide a useful comparative assessment of the effect of land management at the land use type scale. However, the majority of previous studies on denitrification potential in relation to the effect of land use type in the UK have been focused on a single land use type, for example, either in riparian,[18, 19] forest,[20] grassland[21] or arable soils[22, 23] with the exception of Sgouridis *et al.*.[24] To our knowledge, no previous study has evaluated the impacts of different land use types (natural to semi-natural) on soil denitrification potential under the same climatic and edaphic conditions. Moreover, most studies have addressed within site variability of denitrification within single catchments, thus lacking replication of land use types across catchments. Therefore, studies involving replication of land use types within representative catchments and across time could further improve our understanding of the impact of land management on denitrification potential.

Highly organic soils, such as peats, which are regularly at, or exceed field capacity, under natural conditions are generally nutrient limited and their denitrification potential has been shown to be primarily limited by the availability of nitrate.[25, 26] Compared to peatlands, forest soils, particularly those developed under poorly-drained conditions have been shown to sustain a relatively higher denitrification potential.[15, 27] Among forests, deciduous forests are more potent than coniferous forests in their denitrifier activity[15, 20, 27, 28] mainly due to the naturally occurring differences in nitrate availability,[27, 29] soil water filled pore space (WFPS),[28] the quality of leaf litter[29] and soil organic C.[30] Conversely, the denitrification potential of grazed grassland soils (both improved and unimproved) is rarely limited by nitrate availability due to the anthropogenic N inputs in these ecosystems through management practices.[4] Traditional land management practices (e.g. grazing, fertilisation, mowing, liming) in grasslands have been associated with increased denitrification activity due to the additional supply of reactive N through fertilisation,[31, 32] and the supply of organic C and N through the deposition of urine and faeces during grazing.[8, 33-35] Therefore, there is strong indication for a soil nitrate enrichment gradient (very low to excessive) that may be responsible, among others, for the differences in denitrification activity between natural, semi-natural and intensively managed rural land use types. We hypothesise that the relative magnitude of denitrification activity may be regulated by the gradient of nitrate enrichment between natural, semi-natural and managed rural land use types, as these land uses are generally 'organic C' rich with an average soil moisture regime conducive for denitrification activity.

The main objectives of the present study were: (1) to investigate the relative magnitude of the denitrification potential across rural land use types ranging in soil nitrate concentrations, and (2) to assess the environmental controlling factors of denitrification activity as influenced by land management practices. This study is part of a larger Natural Environment Research Council consortium project on the 'Analysis and simulation of the long-term and large-scale interaction of the C, N and P in UK land, freshwater and atmosphere'.

2. Methods

2.1 Study sites and sampling strategy

The Conwy (area 345 km^2; N. Wales) and the Ribble - Wyre River catchments (area 1145 km^2; NW England) were selected in the present study as representative UK catchments where more than 90 % of land cover consists of natural, semi-natural and managed rural land use types.[10] Moreover, these catchments have been identified as priority catchments by the Natural Environment Research Council (NERC) Macronutrient Cycles Program with the aim of quantifying the scales (magnitude and spatial/temporal variation) of N, C and P fluxes between soil, water and air and the nature of transformations through the catchments under a changing climate and perturbed C cycle.

In the Conwy catchment, four land use types (C-PB = peat bog; C-UG = unimproved grassland; C-IG = improved grassland; C-MW = mixed woodland) were selected for soil sampling between the villages of Ysbyty Ifan and Ffestiniog (Figure 1a). This upper 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}.[36] The C-PB (52°59'59" N, 3°48'13" W) is predominantly a *Calluna vulgaris – Eriophorum vaginatum* peat bog with localised areas of *Erica tetralix – Sphagnum papillosum*. The peat bog is currently protected as part of the 2750 ha of Site of Special Scientific Interest (SSSI) land included within the National Trust's Ysbyty Estate. It is current management policy that no area of the peat bog is burnt, the grazing regime is light, meaning less than one sheep per hectare, and the bog is not affected by recreational uses.[36] The C-UG (53°0'03" N, 3°46'30" W) is a transitional area between *Calluna vulgaris – Eriophorum vaginatum* peat bog and *Nardus stricta – Juncus* *squarrosus* acid grassland, while *Sphagnum papillosum* and other bogmoss species are locally common. It belongs to the Blaen-y-Coed farm, grazing activity is restricted to small sheep numbers and the grasslands are not mowed, limed or fertilized (E. Ritchie, pers. comm.). The C-IG (52°59'82" N, 3°46'06" W), also within the Blaen-y-Coed farm, is characterised by seasonally waterlogged cambric stagnogley soils, while the dominant grass species are *Agrostis capillaris* and *Festuca rubra* (Nat. Trust, pers. comm.). This improved grassland is grazed perennially by both sheep and cattle, while fertiliser (range 100 – 200 kg N ha^{-1}) and manure are applied twice per year during spring and summer months (E. Ritchie, pers. comm.). The C-MW (53°0'30" N, 3°45'62" W) is characterised by typical brown podzolic soils that are shallow and well drained, while bare rock is locally visible and steep slopes are common. The dominant tree species are *Acer pseudoplatanus*, *Fraxinus spp.*, *Pseudotsuga menziesii* and *Larix decidua.* This mixed mature woodland belongs to the National Trust's Ysbyty Estate and since its plantation in 1922 has been unmanaged (Nat. Trust, pers. comm.).

In the Ribble-Wyre catchment, five land use types (R-UG = unimproved grassland; R-IG = improved grassland; R-HL = heathland; R-CW = coniferous woodland; R-DW = deciduous woodland) were selected between the village of Quernmore to the east of Lancaster city and the Gisburn forest in east Lancashire (Figure 1b). The dominant soils in the area have been described as stagnopodzols to stagnohumic gleys and the altitude ranges from 260 to 290 m above sea level and the average rainfall is 1693 mm yr^{-1}.[37] The R-UG (54°0'24" N, 2°41'69" W) and R-IG (53°59'99" N, 2°41'79" W) land uses are both within the Low Moorhead farm and are described as wet grassland (dominant species *Juncus effusus* and *Juncus acutiflorus*) and dry grassland (dominant species *Agrostis capillaris* and *Festuca rubra*) respectively, while the land management practices are analogous to the ones described for the unimproved and improved grasslands in the Conwy catchment. The R-UG was fertilised with N in the last decade once and has not been fertilised since, while it is being mowed twice per year (R. Rhodes, pers. comm.). The R-HL (53°58'35" N, 2°34'55" W) is a *Calluna vulgaris* dominated heathland with localised patches of *Sphagnum spp.*, which is privately owned, managed as a grouse moor, grazed by sheep at low densities, while some recreational activities such as hiking are also allowed (Abbeystead Est., pers. comm.). The R-CW (53°59'32" N, 2°23'51" W) and R-DW (54°0'03" N, 2°23'58" W) are both located in the Gisburn forerst, which was established in 1955 on poorly drained former sheep grazing lands and the soils have never been fertilised.[37] The R-CW is a coniferous woodland (dominant species *Picea abies* and *Pinus sylvestris*) on flat topography, while the R-DW is an ancient deciduous woodland (dominant species *Alnus glutinosa, Fraxinus spp.* and *Quercus petraea*) on gently sloping organic rich soils.

To investigate the denitrification potential, five sampling plots were selected randomly in each land use type in the two catchments using a random number table. Three soil cores (0 – 10 cm depth; 5 cm diameter) were collected from each sampling plot using a hand auger. Additional intact soil cores (50 mm I.D., 10 cm long), one per sampling point, were collected for the determination of soil bulk density. In the case of C-PB and R-HL the top 5 cm of live plant material was removed before coring and the core was collected from 5 - 15 cm depth for both the denitrification potential and bulk density determinations. All land use types were sampled in January 2013 and the same sampling procedure was repeated in July 2013, apart from the R-CW which was sampled once in January 2013 and the R-DW that was sampled in July 2013 only. The three cores from each sampling plot were bulked together to form a composite sample; the samples were transported to the laboratory on ice and stored at 4 °C overnight. The next day visible stones and roots were removed manually and the soils were homogenised by manual mixing before laboratory analysis.

2.2 Soil properties

The main physico-chemical soil properties: dry bulk density; porosity; WFPS; pH; soil moisture and organic matter (by Loss on Ignition) contents were analysed according to established methods.[38-40] The gravimetric soil moisture content was reported as per wet basis for comparison purposes between very moist organic and mesic mineral soils.[41] Field moist soils (10 g) were extracted at a ratio of 5:1 with 50 mL 2M KCl and 50 mL deionised water for the determination of inorganic nitrogen species, nitrate (NO_3^-) and ammonium (NH_4^+), and dissolved organic carbon (DOC), respectively.[38] The soil slurries were continuously shaken on a reciprocating shaker at 200 rpm for 1 hour before being centrifuged at 4000 rpm for 20 minutes followed by filtration into 20 mL scintillation vials through a No. 42 Whatman filter paper and were frozen until analysis. The analysis for NO_3^- and NH_4^+ was performed on a Lachat flow injection analyser (Hach, Colorado, USA) according to standard colorimetric techniques.[42] The limit of detection for NO_3^- was 0.03 mg N L^{-1} and for NH_4^+ 0.01 mg N L^{-1}, the samples were blank corrected, while the precision as a relative standard deviation (RSD) was < 5 %. DOC analysis was performed on a HiPerTOC Carbon analyser (Thermo Electron Corp., Delft, The Netherlands) following a standard high temperature combustion method at 1000 ^oC with non-purgeable organic carbon.[43] Standards of 10, 20, 50 and 100 mg L^{-1} C concentrations prepared from anhydrous potassium hydroxyl phthalate ($KHC_8H_4O_4$) were used for calibration. The samples were blank corrected, while the precision of the HT_NPOC method was assessed by measuring 5 repeat injections and the RSD was < 5 %. Soil mineral N and DOC contents are expressed on an area basis for the upper 10 cm of soil (i.e. g N or C m^{-2}) because of the high variability of soil bulk density among sites, which made it difficult to compare results on dry soil weight basis.[15] Moreover, the soil physico-chemical properties as well as the process rates described below will be used for modelling denitrification as part of the larger NERC consortium project and therefore, results reported at an areal basis are more appropriate. Following treatment of mineral soils with hydrogen peroxide and of organic soils with loss on ignition (to remove organic matter), the absolute particle size distribution was determined with optical laser diffraction[44] using an LS 13320 Coulter Counter Particle Size Analyser (Beckman Coulter Corp., Hialeah, FL, US).

2.3 Denitrification, net nitrification and microbial respiration potential measurements

The denitrification potential was estimated using the acetylene inhibition technique (AIT) as in Ullah and Faulkner[6] by amending the slurries with and without nitrate with the aim of measuring the DP of soils in the different land use types. By this approach the DP represents the active denitrifier enzymes in the soil at the time of sampling and is more representative of field denitrification activity.[45] We attempted to address the shortcomings of the AIT,[46] specifically the incomplete inhibition of N_2O reduction to N_2 and the decomposition of pure C_2H_2 by C_2H_2-degrading microbes, through thorough mixing of the slurries after C_2H_2 addition, by increasing the proportion of C_2H_2 in the headspace and by applying short incubation times. Subsamples (10 g field moist soil) from each composite soil sample were weighed into duplicate serum bottles (100 mL) and 30 mL of 3.33 mg NO_3^- L^{-1} solution was added to one set of aliquots, while the other set of aliquots received 30 mL of deionised water (control). The bottles were capped with butyl rubber septa and purged with O_2-free N_2 gas for 30 minutes to induce anaerobic conditions before replacing 15 % of the headspace with pure C_2H_2 to block the conversion of N_2O to N_2 gas. Subsequently, the bottles were wrapped in aluminium foil and transferred onto a reciprocating shaker for thorough mixing at 200 rpm at room temperature. Gas samples were collected from the headspace at 0, 3 and 6 hour duration with a syringe and transferred in to pre-evacuated borosilicate glass vials (3 mL, Exetainer vial; Labco Ltd., High Wycombe, UK). Gas samples were analysed for N_2O on a GC-μECD (7890A GC Agilent Technologies Ltd., Cheshire, UK) and after applying a Bunsen absorption coefficient of 0.54 at ~25°C for accounting for dissolved N_2O, rates of N_2O (proxy for total DP) were estimated based on linear change of concentration in the headspace over time and expressed as mg N_2O-N m^{-2} h^{-1} up to 10 cm depth.

For the estimation of the net nitrification potential of soils, 10 g of field moist soil from each composite sample was weighed into 250 mL plastic bottles, which were then capped with perforated parafilm, to maintain aerobic headspace conditions, and the samples were incubated in the dark at room temperature for 21 days. The gravimetric moisture content was checked weekly in each bottle and adjusted to its initial value by adding deionised water. At the end of the incubation, the soil samples were extracted for the determination of NO_3^- as per section 2.2. The net-nitrification potential was calculated as the difference in soil nitrate content between 0 and 21 days and expressed as mg NO_3^--N m^{-2} h^{-1} up to 10 cm depth as in Ullah and Moore [29].

Several studies have used the mineralisation rate of organic carbon under anaerobic conditions as a proxy for the labile organic carbon fraction available to denitrifiers for nitrate reduction.[6, 24, 47] In the present study, we measured the microbial respiration potential through the evolution of CO_2 during the six hour incubation of the NO_3^-- amended slurries, which represents the fraction of labile organic carbon available under non-limiting nitrate conditions to the denitrifier enzymes during the same incubation period. The gas samples that were collected during the denitrification experiment were also analysed for CO_2 on a GC-FID (7890A GC Agilent Technologies Ltd., Cheshire, UK) and after applying a Bunsen absorption coefficient of 0.75 at ~25°C for accounting for dissolved CO_2, potential microbial respiration rates were estimated by linear regression between 0, 3 and 6 hours and expressed as g CO_2 m^{-2} h^{-1} up to 10 cm depth.

2.4 Statistical analysis

Prior to any statistical tests the data were analysed for normality and homogeneity of variance with the Kolmogorov-Smirnov test and the Levene statistic respectively and log-transformation was applied where appropriate. Principal Component Analysis (PCA) was used to explore the combinations of soil physico-chemical properties, 'principal components', which are likely to provide the maximum discrimination between individual plots.[48] Comparisons between experimental treatments and between sampling seasons were performed with unpaired t-test. One-Way ANOVA combined with the Least Significant Difference (LSD) *post hoc* test were performed for comparing the variance between groups and for the assessment of inter-sample group differences respectively. The variance of those samples that were not log-normally distributed was tested with the non-parametric Kruskal-Wallis test. Non-parametric Spearman correlation was used instead of Pearson correlation between not normally distributed variables. Multiple stepwise linear regression was used to explore the factors controlling DP within each land use type. Model outputs of predictor variables were tested for multicollinearity using the variance inflation factor (VIF), and residual autocorrelation using the Durbin-Watson's test. All statistical analyses were performed using SPSS® 19.0 for Windows (IBM Corp., 2010, Armonk, NY).

3. Results

3.1 Variation in soil physico-chemical properties across land use types

Principal Component Analysis (PCA) of soil physico-chemical variables across the eight land use types (n = 75), with the exception of the coniferous woodland (n = 5) where some data were not available, was employed to separate land use type groups based on the maximum variance explained by their soil properties. The PCA identified two components with eigenvalues larger than 1, which together explained 84 % of the total variance within the data set. The soil moisture and organic matter contents correlated significantly ($p < 0.01$) with the positive axis of the first principal component (PCA1), whilst nitrate correlated with the negative axis of PCA1, explaining 56 % of the observed variance in the overall data. Soil ammonium and DOC showed higher correlation coefficients ($p < 0.01$) with the positive axis of the second principal component (PCA2), which explained an additional 28 % of the variance in the dataset (Figure 2).

Cluster centroids (average score on each component, with standard errors) for each land use type are presented in Figure 3. The samples from individual field plots were grouped into land use types mostly along PCA1, where the sites C-PB, C-UG and R-HL formed a distinct group hereafter called organic soils (OS) as these are characterised by high soil organic matter and moisture and low bulk density, clay, nitrate and microbial respiration (CO_2) potential (Table 1). At the other end of the spectrum (negative axis of PCA1) the sites C-IG and R-IG clustered together, which are hereafter called the improved grassland (IG) land use type. In terms of soil properties, IG is closely associated with the mixed woodland (MW) in the Conwy catchment. Both of these (IG and MW) land use types are characterised by relatively higher bulk densities and low organic matter; however, the MW had a significantly lower WFPS and higher nitrate content ($p < 0.05$) compared to the IG (Table 1). The site R-UG formed a distinct land use, named as semi-improved grassland (SIG). The SIG is an intermediate land use type between OS and IG in the sense that it resembles OS in having high WFPS and DOC, but has significantly higher nitrate and clay contents than the OS. Moreover, the pH of the SIG was similar to that in the IG (Table 1), mainly due to it being fertilised about 10 years ago and often mowed. Finally, two more woodland types were identified, the coniferous woodland (CW), sampled in January 2013 (R-CW), and the deciduous woodland (DW) sampled in July 2013 (R-DW site). The DW and CW were both wetter and more organic matter rich compared to the MW, whilst they differed from each other in that the DW had significantly higher pH and nitrate than the CW. CW is not represented in Figure 3 as it was excluded from PCA due to the non-available DOC data.

3.2 Denitrification and nitrification potential

Averaged denitrification potential (average of January and July 2013 DP rates) varied between 0.02 and 63.3 mg N m^{-2} h^{-1} across the different land use types. The IG showed the highest DP, followed by the SIG, whilst the OS and the woodland land use types were significantly lower (ANOVA; $F = 15.6$, $df = 5$, $p < 0.01$) from the IG and SIG but not different from each other (Figure 4a). A similar trend of lower DP in OS and MW followed by DW, SIG and IG (ANOVA; $F = 49.4$, $df = 4$, $p < 0.001$) was observed in July 2013, whilst in January 2013 differences of DP between land use types were less prominent (ANOVA; $F = 5.52$, $df = 4$, $p < 0.01$), but significant nevertheless between natural (OS, CW, MW) and semi-natural (SIG and IG) land use types.

When the DP rate of each land use type measured in January and July was compared for a seasonal effect using a t-test (Figure 4b), no significant difference in DP rates was observed in the case of IG and SIG (IG: $t = 1.25$, $df = 18$, $p > 0.05$ and SIG: $t = 1.18$, $df = 8$, $p > 0.05$). On the other hand, DP was significantly higher in January compared to the July DP rates of the OS and MW (OS: $t = 3.07$, $df = 28$, $p < 0.01$ and MW: $t = 3.51$, $df = 8$, $p < 0.01$). Evaluation of a seasonal effect was not possible in case of CW, which was sampled once in January 2013 and the DW once in July 2013.

When amended with additional nitrate during the experimental procedure, only the OS exhibited a significant increase in DP compared to the DP of the un-amended control soils (OS: t-test; $t = 2.92$, $df = 58$, $p < 0.01$), whereas a similar effect of nitrate amendment was observed for CW albeit not statistically significant (p >0.05). In the case of IG, SIG, DW, and MW non-significant differences of DP in response to nitrate amendment were observed (Figure 5a). It is interesting to note that the DP of the nitrate amended OS was still significantly lower than the DP of IG and SIG with and without nitrate amendment (p < 0.01).

The mean net-nitrification potential ranged between 0.1 ± 0.04 and 4 ± 0.69 mg N m^{-2} h^{-1} (Figure 5b). The MW showed the highest net-nitrification potential, whilst the OS and CW were the lowest, followed by intermediate rates in the SIG and IG (ANOVA; $F = 8.7$, $df = 5$, $p < 0.01$). A similar trend was observed when only the July 2013 samples were considered (ANOVA; $F = 9.9$, $df = 4$, $p < 0.01$), while in January 2013, only the MW displayed significantly higher net-nitrification potential (ANOVA; $F = 7.8$, $df = 4$, $p < 0.01$).

3.3 Factors controlling denitrification across and within land use types

Denitrification potential across all land use types (n = 80) correlated positively with the bulk density, pH, nitrate and clay contents and the microbial respiration potential (Table 2). A significant positive correlation was found between DP and net-nitrification potential, when the MW land use, where nitrification was the highest but DP was at the lowest range, was excluded from the correlation analysis (Spearman: $r = 0.32$, $p < 0.05$, $n = 67$). Multiple linear regression (MLR) of log-transformed DP rates on soil properties was also performed to identify the factors controlling DP within each land use type. In case of the OS, the combination of DOC and bulk density explained 41 % of the variance in the DP rates (Table 3). Water-filled pore space and DOC explained 89 % of variability in DP of the MW. Finally, bulk density and clay content accounted for 72 % of the variability in DP of the SIG land use type. The MLR in case of the IG (n = 20) did not show any significant linear relationship between DP and soil environmental variables, while MLR was not performed in the case of CW and DW, due to the low sample number (n = 5).

4. Discussion

The observed rates of DP were lower in the OS and forest soils (CW, DW and MW), followed by the semi-improved grassland (SIG) and the improved grassland (IG) indicating a difference in denitrification activity between unmanaged/ low nitrate content versus managed/ high nitrate content land use types. Nitrate correlated positively with DP, even though weakly, supporting partially our hypothesis that differences in soil nitrate concentration between the OS, forest and grassland soils regulated the extent of DP, whilst suggesting that the relative importance of other controlling factors (e.g. organic C, moisture, bulk density, etc.) within each land use type may be responsible for the observed differences in DP. A similar influence of nitrate enrichment of wetlands in Louisiana on denitrification activity was observed by Gardner and White.[49] The presence of high soil nitrate due to high nitrification potential observed in the MW and DW soils was an exception, where DP appeared limited by their low soil moisture regimes, among other factors (details below). The term organic soils was applied in this study based on the PCA analysis to include soil samples from a peat bog, a heathland and an acid grassland, which are generally considered having low denitrification activity due to their inherent low nitrate availability in excess of plant and microbial metabolic demands.[25] The 10-fold increase of DP in the nitrate-amended compared to the un-amended soils verified that nitrate availability was indeed limiting denitrification in OS, which is in agreement with the findings of Pinay *et al.*; Hayden and Ross; Francez *et al.* and Urban *et al.*.[15, 25, 26, 50] However, an episodic increase in nitrate availability (such was the case of our treatment) may not translate to high field scale denitrification rates since the *in situ* abundance of denitrifying enzymes can be low due to low nitrate content and competition between plants and microbes for the available N. For example, Francez *et al.*[26] have shown that a longer term period (up to 45 days) of excessive N enrichment (above the current atmospheric N deposition rates for Europe) is needed to stimulate *de novo* synthesis of denitrification enzymes in peat soils with consequent exponential increase in DP rates. This is further supported by the observation that the OS responded to nitrate amendment with high DP rates, yet these rates were 2.4 and 9 times less than the DP of nitrate rich SIG and IG soils, respectively.

Soils under the three forest types maintained lower denitrifier activity than the grasslands (SIG and IG). When amended with nitrate, the DP rates of the forest soils were similar with the exception of CW; however, the DP of un-amended control soils collected from DW and MW was 9 and 23 times higher than the DP of CW soils, which is consistent with the findings of Ullah *et al.*[51] The denitrification potential rates of the three forest soils were in the range reported for forest soils dominated by American beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*) and eastern hemlock trees (*Tsuga Canadensis*).[52] Low soil nitrate in the CW (below the limit of detection) seems to have maintained a relatively lower denitrifier activity which increased, albeit not statistically significant, when amended with nitrate, while low WFPS was limiting DP in case of MW and DW (WFPS < 60 %). Several studies have shown limitation of denitrification activity in forest soils by low WFPS,[20, 28, 52, 53] which is more likely due to the limited adaptation of the microbial community to anaerobic conditions and the dynamics of NO_3^- and NO_2^- reductases.[20] Atmospheric N deposition of 0.5 to 3.5 g N m^{-2} yr^{-1} in the UK[54] across the selected OS and forest soils may have not yet exceeded the plant and microbial metabolic N demands to support a higher denitrifier activity like that of SIG and IG land use types.[5, 49]

In contrast to the OS and forest soils, the land management regimes applied in both the SIG and IG grasslands supply additional anthropogenic reactive N, on top of the atmospheric N deposition to support higher denitrifier activity. These additional sources of reactive N input into grasslands seem to have supported a markedly high DP with no indication of nitrate limitation (Figure 5a). Although fertilisation has ceased at the SIG land use since the previous decade, the IG land uses are currently fertilised at an annual rate between 100 - 200 kg N ha^{-1}, a fertilisation intensity, which was shown to increase denitrification activity and lead to high N_2O emissions from grasslands in the UK.[55] Both the SIG and the IG land use types are grazed throughout the year and grazing has been related to increased denitrification rates, because of the additional inputs of organic C and N through the deposition of urine and faeces.[31, 56] Moreover, the density of grazing (i.e. number of animals per hectare) as well as the grazing species can also have an effect on denitrification activity.[35] The SIG is grazed exclusively by sheep at low densities while the IG sites are grazed by both sheep and cattle at higher densities especially during the summer months when fertiliser application also occurs. The combinations of higher grazing intensity, N fertilisation and thus high soil nitrate in the IG seem to have maintained higher DP rates compared to the SIG land use type. Our results suggest that nitrate availability is limiting denitrification activity in the inherently N poor natural ecosystems such as peatlands, heathlands, acid grasslands and forests; whilst in SIG and IG the DP is uncoupled from nitrate availability control due to the land management practices that supply additional reactive N.

Additional evidence for the importance of nitrate supply in controlling denitrification activity is given by the observation of significant differences in the net nitrification potential among land use types (Figure 5b). Nitrification may had most likely been restricted in the OS by anoxia due to high moisture and also low pH,[25] and the latter likely had a similar effect on nitrification rates in the CW soils as well.[57] We did not measure the soil C:N ratios in the selected land use types, but in general OS and forest soils, particularly coniferous forest soils have high C:N ratio (> 25:1) that often limits net nitrification rates[25, 29, 58] compared to those in SIG and IG land uses. In case of the MW, the lower WFPS (45%) probably favoured nitrification over denitrification,[29, 40, 52] which explains the significantly higher nitrification potential.

Other than soil nitrate, the availability of organic C was an additional factor responsible for the observed differences in the DP. Microbial respiration as a proxy for the availability of labile organic C, correlated significantly with DP (Table 2). Several studies have shown a positive relationship between the labile fraction of the available organic C and denitrification activity in a wide range of land use types.[6, 24, 47] In the OS and MW land uses, the multiple linear regression analysis identified DOC as one of the controls of DP (Table 3). DOC content in soil is regarded as a surrogate indicator of microbially available organic carbon substrate supporting denitrification[59] and often limits denitrification activity in well-drained forest soils.[60, 61] In peat soils the supply of readily hydrolysable organic C can have a significant positive effect on denitrification activity,[62] since the waterlogged, low oxygen and low pH conditions limit C decomposition and/or availability.[50] This view is also supported by our results of microbial respiration potential, which was lower in the OS compared to the IG. The microbial respiration rates in the OS observed in this study were comparable to the rates measured in an un-drained monolith fen ecosystem in northeast Poland.[63] Forest soils and the managed grasslands exhibited similar microbial respiration potentials, which were higher than the values reported in a N-rich floodplain grassland in England, where the lability of organic carbon was identified as the main controlling factor of denitrification.[24] Land management activities such as grazing and manure application have been shown to increase both the availability[31, 56, 64] and the lability of organic C[65] leading to enhanced denitrification activity. Mowing, which is applied to the SIG land use twice per year, is another land management activity that may have supported enhanced denitrifier activity as mowing has been shown to result in the release of DOC and mineral N due to the short life-cycle of plants in mown grasslands.[56, 64] Thus, the lability of organic C under anaerobic conditions appear to have contributed to the differences in the DP across the land use types in addition to the availability of nitrate, particularly, between the OS and IG soils.

In addition to the proximal regulators of denitrification (e.g. organic C, N and soil moisture), distal factors (e.g. soil pH, bulk density and clay content) that directly or indirectly affect denitrification[4] differed across the selected land use types. Land management such as liming in grasslands is aimed at raising soil pH for higher biomass productivity.[54] Thus, raising pH could also have implications for both nitrification and denitrification activity as low soil pH has been identified as one of the possible factors limiting nitrification in peat and coniferous forest soils,[25, 57] whilst denitrification is generally slower under acidic soil conditions,[66, 67] which is commensurate with the positive correlation between DP and pH across the land use types.

Soil bulk density and clay percentage correlated positively with the DP across all the land use types and were also highlighted as additional controlling factors in the OS and SIG land uses (Table 3). Herbivore trampling in clayey soils has been related to enhanced denitrification rates due to compaction resulting in an increase in bulk density and decrease in porosity, which subsequently creates anoxic microsites in soils.[68] Clay and silt content over 65 % has been associated with high soil denitrification rates in a pan-European study by Pinay *et al.*[69] The OS had the lowest bulk density and clay content than the forest and grassland soils and this difference appears to be driven by the land cover (e.g. vegetation type) and land management (compaction). This suggests that land management in grasslands, in particular, may have further supported higher denitrifier activity by influencing distal controls of denitrification such as bulk density, clay content and pH in conjunction with the relatively higher soil nitrate content.

Seasonal differences in the DP have been attributed to the effect of temperature and antecedent WFPS conditions on denitrification enzyme activity.[6] In our study, seasonal difference in DP was only observed in the OS and MW land use types (Figure 4b), whilst results are inconclusive with respect to the CW and DW land uses that lacked seasonal replication. The lower DP observed in summer compared to winter for the OS could most likely be attributed to the increased competition for nitrate between plants and the denitrifying population[50] rather than changes in the WFPS, which was > 60 % in both summer and winter at the time of sampling. Rubol *et al.,*[70] showed no significant change in N_2O emissions from peatland soils with WFPS ranging between 60 and 100 %. In contrast, the WFPS in the MW in July 2013 was 30 % compared to 60 % WFPS measured in January 2013, and thus low antecedent soil moisture conditions may have negatively affected the DP in this land use type.[22] In case of the grassland soils, the DP rates measured in January and July were not significantly different. Any possible seasonal effect on the relative magnitude of DP in the grasslands may have been minimal relative to the range of DP observed under no nitrate limitations. Moreover, the average DP trend across land use types (Figure 4a) is most likely influenced by the July 2013 sampling as indicated by the ANOVA results for each sampling season; however a significant difference in DP between unmanaged (OS, CW, MW) versus managed (SIG & IG) land use types is also evident in the January 2013 results. This finding gives us confidence in the reported DP differences between land use types, but we acknowledge that further studies are needed to elucidate any seasonal impacts on denitrifier activity within individual land use types.

Our results showed that denitrification activity can be significantly enhanced by the enrichment of soils with reactive N in rural catchments through the prevailing land management practices and this can have economic and environmental implications. From an agricultural perspective, denitrification results in the loss of valuable N fertiliser with economic consequences for the farming industry; whilst from an environmental perspective increased denitrification poses the threat of increasing N_2O emissions, which is of concern due to the high global warming potential of N_2O (~ 300 times greater than CO_2) and its involvement in the breakdown of stratospheric ozone.[71] As agriculture is considered responsible for 79 % of the anthropogenic N_2O emissions in the UK,[72] it becomes evident that land use practices in managed rural ecosystems will need to be adapted in order to minimise the accumulation of reactive N in soil to restrict fertiliser loss via denitrification, and where denitrification is inevitable, maximise the emission of N_2 rather than N_2O.[4] In contrast, organic and forest soils in the two catchments exhibited denitrification potential rates representative of N limited systems.[25, 58] Denitrifier activity in OS and forest land use types may be enhanced further than the observed DP rates, if exposed to excessive reactive N loading beyond microbial and plant uptake demands.[25, 26] The determination of DP was undertaken under optimum laboratory conditions of temperature, moisture, and anoxia; therefore, the results cannot not be directly extrapolated to estimate field denitrification rates. Currently, monthly measurements of *in situ* denitrification rates using the ^{15}N flux methods[73] is underway in the same land uses in an effort to quantify annual denitrification rates and to validate and evaluate the controls of denitrification reported in this study.

5. Conclusion

The results show significant difference in the denitrification potential between unmanaged/ low nitrate content versus managed/ high nitrate content land use types , where nitrate availability is affected by both the natural variability in N cycling and also land management practices. The low DP of OS was primarily controlled by the inherently low nitrate due to low nitrification potential, whilst the quality of organic carbon and pH seem to have exerted additional controls. The unmanaged forest land use types, where atmospheric N deposition is the sole source of additional reactive N input, exhibited DP rates that were lower than the SIG and IG land use types mainly due to low nitrification potential, low pH, low WFPS, or a combination of these controlling factors according to forest type. Conversely, the SIG and IG land use types exhibited the highest DP rates with no apparent nitrate limitation. The relatively high net nitrification potential together with additional reactive N inputs due to atmospheric N deposition and land management practices such as fertiliser application, mowing, and grazing seem to have supported higher denitrifier activity in the SIG and IG land use types. The results suggest that land management practices can have significant impacts on the biogeochemical controls of denitrification, and thus need consideration when modelling denitrification across large spatio-temporal scales and/or predicting the response of denitrification to land use change.

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Figure Legends

Figure 1: Location of study sites in: (a) Conwy River catchment and (b) Ribble – Wyre River catchment. C-PB = Peat Bog; C-UG = Unimproved Grassland; C-IG = Improved Grassland; C-MW = Mixed Woodland; R-UG = Unimproved Grassland; R-IG = Improved Grassland; R-HL = Heathland; R-CW = Coniferous Woodland; R-DW = Deciduous Woodland.

Figure 2: Correlation bi-plot from the PCA analysis on soil physico-chemical variables. MC; Moisture content, OM; Organic matter content.

Figure 3: Correlation bi-plot from the PCA analysis with cluster centroids from the land use types in the Conwy and Ribble - Wyre River catchments.

Figure 4: Mean denitrification potential in the different land use types of the Conwy and Ribble - Wyre River catchments: (a) Averaged denitrification potential between January and July 2013 measurements, (b) Denitrification potential separated between January and July 2013 measurements. OS = Organic Soils; CW = Coniferous Woodland; MW = Mixed Woodland; DW = Deciduous Woodland; SIG = Semi-Improved Grassland; IG = Improved Grassland. Comparison of denitrification potential (DP) between land use types performed with ANOVA, while the DP between seasons was compared with unpaired t-test. Significant differences indicated with different lower case letters. Error bars indicate standard error (SE).

Figure 5: (a) Mean denitrification potential of soils amended with NO_3^- and un-amended soils (control), (b) Mean net-nitrification potential in the different land use types of the Conwy and Ribble - Wyre River catchments. OS = Organic Soils; CW = Coniferous Woodland; MW = Mixed Woodland; DW = Deciduous Woodland; SIG = Semi-Improved Grassland; IG = Improved Grassland. Comparison of denitrification potential between amended and un-amended soils performed with unpaired t-test, while the net-nitrification potential between land use types was compared with ANOVA. Significant differences indicated with different lower case letters. Error bars indicate standard error (SE).

Table 1: Soil physico-chemical properties in the six land use types in the Conwy and Ribble-Wyre River catchments. 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 the Kruskal-Wallis test. n/a: Data not available; < LOD: Below the limit of detection.

	Organic soils (n = 30)	Coniferous woodland (n = 5)	Deciduous woodland (n = 5)	Mixed woodland (n = 10)	Semi-improved grassland (n = 10)	Improved grassland (n = 20)
Bulk Density (g cm^{-3})	0.09 (0.01)^{a}	0.33 (0.12)^{bd}	0.39 (0.02)^{bcd}	0.50 (0.06)^{cd}	0.34 (0.05)^{b}	0.45 (0.02)^{d}
WFPS (%)	76 (3.3)^{a}	59 (13.8)^{abc}	57 (2.1)^{bc}	45 (5.4)^{c}	74 (6.9)^{ab}	61 (4.9)^{b}
Moisture content (% on w/w)	88 (1.1)^{a}	56 (5.9)^{b}	56 (2.1)^{b}	42 (2.7)^{c}	57 (3.1)^{b}	41 (1.9)^{c}
pH	4 (0.1)^{a}	4 (0.2)^{a}	7 (0.1)^{b}	6 (0.3)^{c}	6 (0.0)^{c}	6 (0.0)^{c}
Clay (%)	7 (0.4)^{a}	n/a	24 (3.1)^{b}	37 (2.1)^{c}	31 (0.6)^{d}	26 (1.5)^{b}
Organic matter (%)	93 (1.3)^{a}	34 (10.9)^{bd}	23 (1.2)^{bc}	15 (1.1)^{c}	38 (5.4)^{d}	19 (1.3)^{c}
DOC (g m^{-2})	11.6 (2.53)^{a}	n/a	6.5 (2.81)^{ab}	3.3 (0.73)^{b}	10.4 (2.95)^{a}	3.4 (0.48)^{b}
Microbial respiration (g CO_2 m^{-2} h^{-1})	0.28 (0.02)^{a}	0.42 (0.12)^{ab}	0.38 (0.03)^{ab}	0.40 (0.07)^{ab}	0.36 (0.06)^{ab}	0.50 (0.05)^{b}
NO_3^--N (g m^{-2})	0.04 (0.007)^{a}	< LOD	0.24 (0.055)^{b}	0.79 (0.068)^{d}	0.23 (0.065)^{b}	0.48 (0.067)^{c}
NH_4^+-N (g m^{-2})	0.28 (0.081)^{a}	0.03 (0.025)^{a}	0.09 (0.012)^{a}	0.17 (0.063)^{a}	0.32 (0.119)^{a}	0.28 (0.082)^{a}

Table 2: Correlation between soil physico-chemical properties and denitrification potential across all the land use types. *r*; Spearman's correlation coefficient, *P*; probability level, n = 80.

	r	*P*
NO_3^--N	0.37	< 0.01
Microbial respiration	0.39	< 0.01
pH	0.49	< 0.01
Bulk Density	0.57	< 0.01
Clay	0.33	< 0.01

Table 3: Multiple Linear Regression Models to assess the factors controlling denitrification potential within land use types. Only the significant relationships are shown. OS = Organic Soils; MW = Mixed Woodland; SIG = Semi-Improved Grassland; DP = Denitrification Potential; BD = Bulk Density; WFPS = Water Filled Pore Space; DOC = Dissolved Organic Carbon

Independent variable	Equation	r^2	*F*	n
Log DP OS	-0.806 + (0.943 x log DOC) + (0.343 x log BD)	0.41	6.03	28
Log DP MW	-4.905 + (2.655 x log WFPS) + (0.972 x log DOC)	0.89	27.0	10
Log DP SIG	0.886 + (0.945 x log BD) + (0.100 x %Clay)	0.72	8.98	10

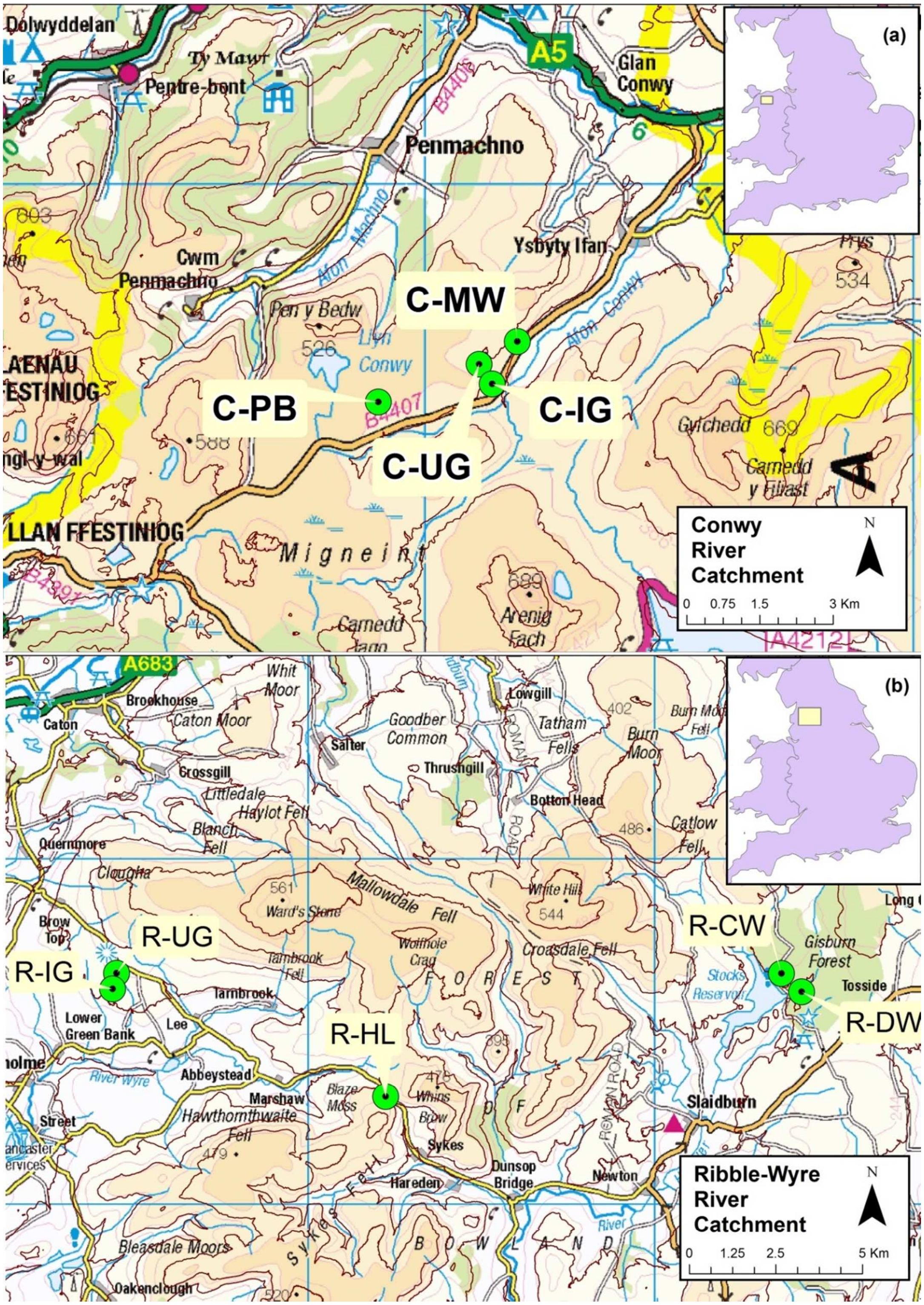

Figure 1: Location of study sites in: (a) Conwy River catchment and (b) Ribble – Wyre River catchment. C-PB = Peat Bog; C-UG = Unimproved Grassland; C-IG = Improved Grassland; C-MW = Mixed Woodland; R-UG = Unimproved Grassland; R-IG = Improved Grassland; R-HL = Heathland; R-CW = Coniferous Woodland; R-DW = Deciduous Woodland.

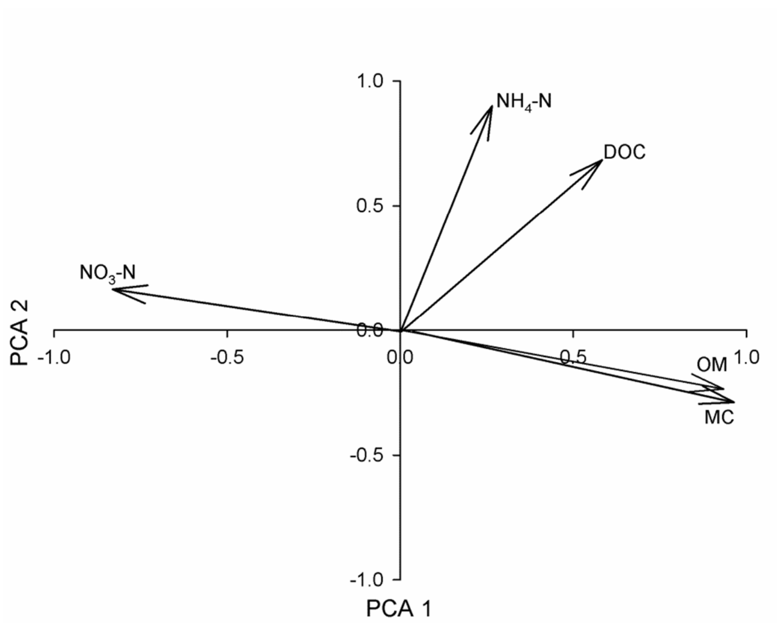

Figure 2: Correlation bi-plot from the PCA analysis on soil physico-chemical variables. MC; Moisture content, OM; Organic matter content.

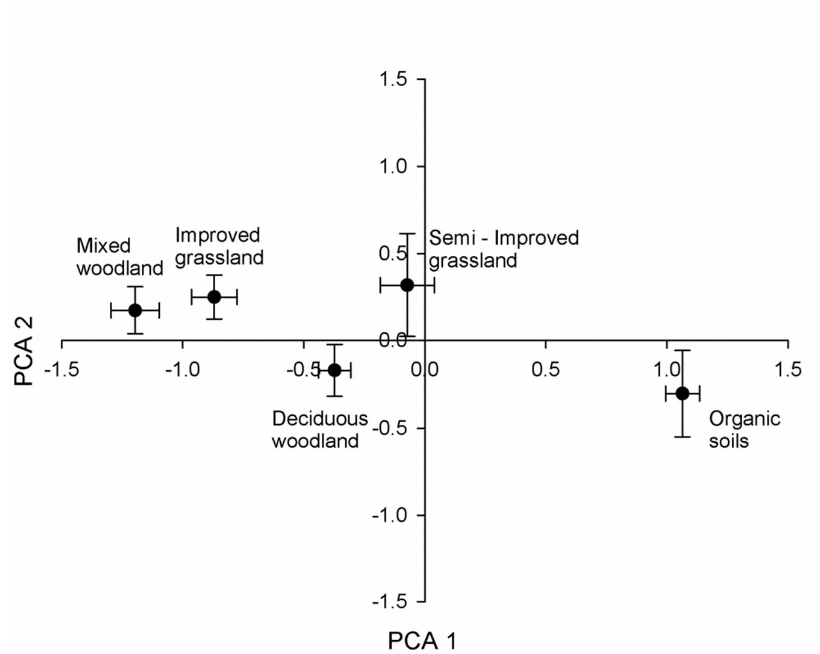

Figure 3: Correlation bi-plot from the PCA analysis with cluster centroids from the land use types in the Conwy and Ribble - Wyre River catchments.

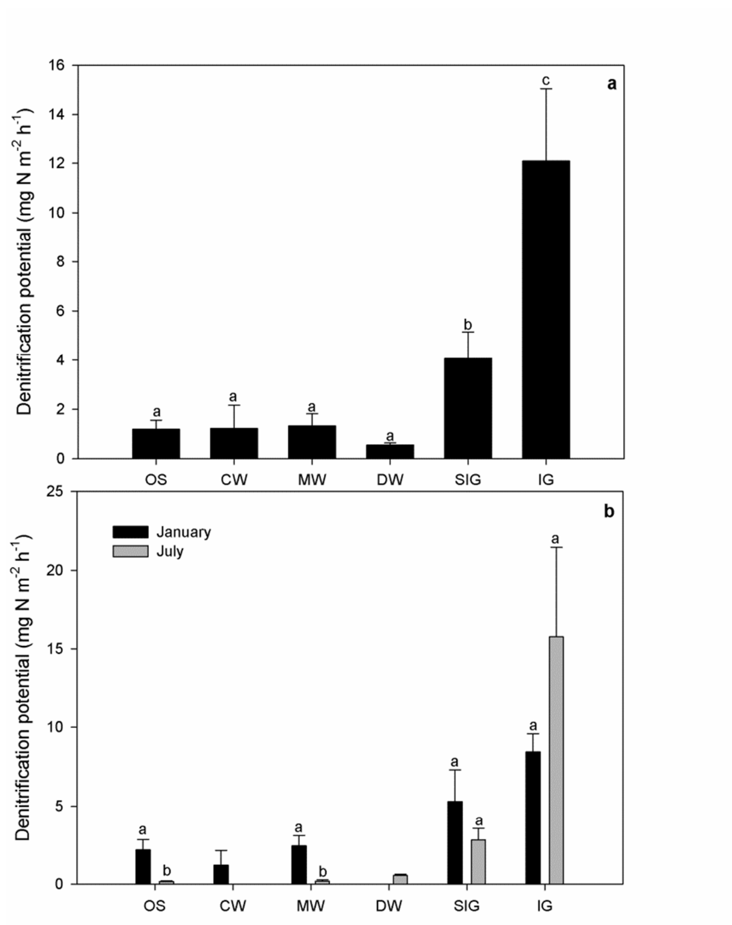

Figure 4: Mean denitrification potential in the different land use types of the Conwy and Ribble - Wyre River catchments: (a) Averaged denitrification potential between January and July 2013 measurements, (b) Denitrification potential separated between January and July 2013 measurements. OS = Organic Soils; CW = Coniferous Woodland; MW = Mixed Woodland; DW = Deciduous Woodland; SIG = Semi-Improved Grassland; IG = Improved Grassland. Comparison of denitrification potential (DP) between land use types performed with ANOVA, while the DP between seasons was compared with unpaired t-test. Significant differences indicated with different lower case letters. Error bars indicate standard error (SE).

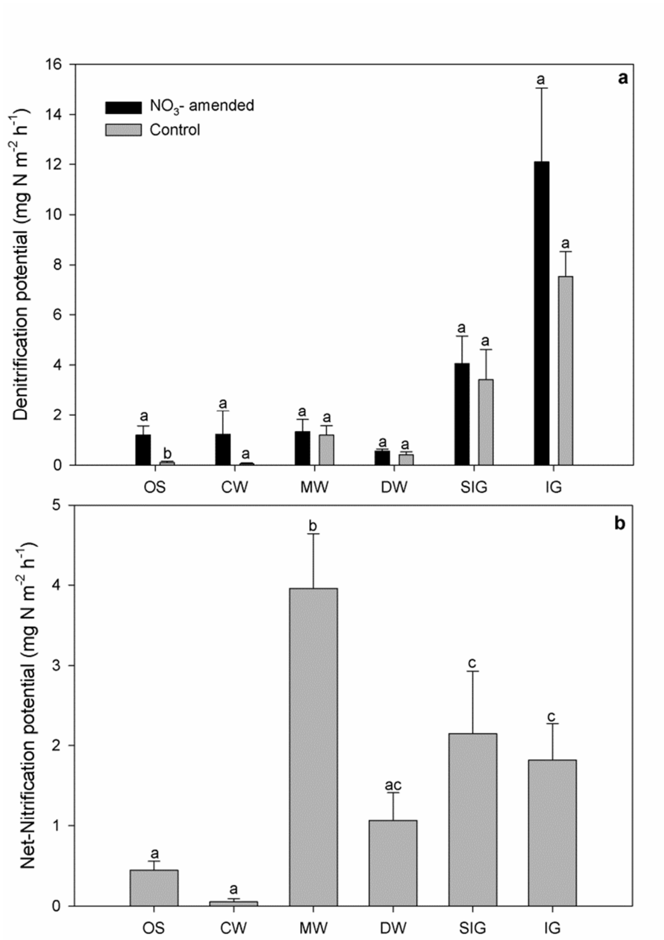

Figure 5: (a) Mean denitrification potential of soils amended with NO_3^- and un-amended soils (control), (b) Mean net-nitrification potential in the different land use types of the Conwy and Ribble - Wyre River catchments. OS = Organic Soils; CW = Coniferous Woodland; MW = Mixed Woodland; DW = Deciduous Woodland; SIG = Semi-Improved Grassland; IG = Improved Grassland. Comparison of denitrification potential between amended and un-amended soils performed with unpaired t-test, while the net-nitrification potential between land use types was compared with ANOVA. Significant differences indicated with different lower case letters. Error bars indicate standard error (SE).