European Journal of Soil Science

European Journal of Soil Science, January 2018, **69**, 48–68 doi: 10.1111/ejss.12504

Effect of long-term drainage on plant community, soil carbon and nitrogen contents and stable isotopic (δ^{13}C, δ^{15}N) composition of a permanent grassland

P. Harris[a], R. Bol[b], J. Evans[c], J. M. B. Hawkins[a], E. R. Dixon[a], K. Wolf[d], J. A. J. Dungait[a], B. Griffith[a], M. Herbst[b], M. S. Dhanoa[a], D. A. Beaumont[a], R. M. Dunn[a] & G. L. B. Wiesenberg[d,e]

[a]*Sustainable Agricultural Sciences, Rothamsted Research, North Wyke, Okehampton, Devon, EX20 2SB, UK,* [b]*Institute of Bio- and Geosciences, Agrosphere (IBG-3), Forschungszentrum Juelich GmbH, Wihelm-Johnen-Strasse, 52425 Juelich, Germany,* [c]*Computational and Analytical Sciences, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK,* [d]*Department of Agroecosystem Research, University of Bayreuth, 95440 Bayreuth, Germany, and* [e]*Department of Geography, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland*

Summary

This study compares data statistically that were collected from both long-term drained and undrained plots to test hypotheses concerning the effect of drainage on plant community, soil total nitrogen (TN), soil total carbon (TC) and stable isotopic (δ^{15}N, δ^{13}C) contents in a permanent grassland. In addition, the effects of soil depth, topography (elevation, slope, aspect and compound topographic index (CTI)) and spatial autocorrelation were taken into account. Data were collected in 2010 at Rowden Moor, North Wyke, Devon, UK, where, for the plots of this study, subsurface drainage was introduced in 1987. The results of a set of six linear mixed models showed that: (i) plant community did not depend on drainage, but on elevation and spatial effects, (ii) both TN and TC not only depended on drainage, but also topography and sample depth, (iii) the TC to TN ratio did not depend on drainage, but on elevation, CTI and sample depth only, (iv) δ^{15}N values did not depend on drainage, but on topography and sample depth and (v) δ^{13}C values depended on drainage together with topography and sample depth. Thus, drainage represented a significant effect for only TN, TC and δ^{13}C. Furthermore, changes in soil physicochemical conditions, following the introduction of drainage in the clay soil 24 years previously, induced a shift in the plant community from a *Lolium perenne* L. dominated grassland with numerous patches of *Juncus* species, towards one with *Lolium perenne* and *Trifolium repens* L.

Highlights

- The effect of drainage on plant community, and C and N cycling on permanent grassland.
- Soil depth, topography and associated spatial effects are taken into consideration.
- Plant community (species diversity) depended on topography and spatial effects only.
- Soil chemistry depended on topography and depth, and N, C and δ^{13}C also depended on drainage.

Introduction

Global climate change is projected to change seasonality and the intensity of rainfall patterns substantially (i.e. more extreme events), which is of particular importance for large areas across Europe where such climatic events can have a strong influence on agricultural practices (IPCC, 2007). Drainage enables improved management of previously wet or waterlogged soil. However, the resulting alterations of the carbon cycle with global climate change and associated feedback from the drainage of land should be considered carefully (Lal, 2004). The artificial removal of water improves growing conditions of plants, promotes mineralization of soil organic matter (SOM), reduces the risk of soil erosion and floods, and increases agricultural production (Lal, 2004; Herzon & Helenius, 2008).

Correspondence: P. Harris. E-mail: paul.harris@rothamsted.ac.uk

This paper was invited for the 70th Anniversary of the British Society of Soil Science and was received on 9 December 2016; revised version accepted 17 September 2017

In grassland systems, drainage primarily results in a reduced risk of turf damage by cattle or machines and therefore promotes early access of the cattle to the meadow (Morris, 1989). Furthermore, drainage enhances more predictable and sustainable silage production and a timely application of nitrogen fertilizers (Morris, 1989). On the other hand, subsurface drainage is often accompanied by disadvantages such as loss of important wetlands, loss of nutrient and water retention capacity, risk of downstream floods, eutrophication of surrounding waters and reduction of farmland biodiversity (Herzon & Helenius, 2008). Even though drainage has been common practice for many centuries, there has been little research into its long-term effects on a decadal range with respect to changes in biodiversity and the elemental composition of a grassland system, although clear effects have been proposed (Lal, 2004).

In soil, total nitrogen (TN) and organic carbon (TC) contents depend on biomass input and its preservation (Don *et al*., 2009; Schmidt *et al*., 2011); the latter is also related to climatic conditions and microbial activity (Chou *et al*., 2008), management and the ecosystem itself (Schmidt *et al*., 2011). The incorporation of TN and TC into soil also depends on plant species and plant communities, respectively, where incorporation occurs from litter fall of above-ground biomass and below-ground allocation by root residues and exudation (Don *et al*., 2009). It has been suggested that the turnover of SOM in soil is governed mainly by accessibility, depending on the physical preservation of SOM (occlusion within aggregates and adsorption on to minerals) (Dungait *et al*., 2012), microbial activity from substrate supply and climatic conditions (Soussana *et al*., 2004). Contribution of above-ground biomass declines with increasing soil depth, and root contribution and mineralization become more important in contrast, whereas TN and TC contents typically decrease with soil depth (Don *et al*., 2009; Gregory *et al*., 2016). Because of limited soil respiration and therefore decomposition processes in water-saturated soil, TN and TC contents are degraded less than in well-aerated soil (Chou *et al*., 2008).

Stable nitrogen (δ^{15}N) and carbon (δ^{13}C) isotope measurements of soil and plant samples have been used to retrieve information about SOM dynamics in soil processes (Balesdent *et al*., 1987). The δ^{15}N composition in plants and soil depends on the general N cycle and provides information about it (Högberg, 1997). Soil processes such as ammonia volatilization and denitrification lead to enrichment of residual N in ^{15}N (Dixon *et al*., 2010). Furthermore, δ^{15}N values are affected by SOM degradation, N-fixation and N-uptake by plants (Dawson *et al*., 2002).

The δ^{13}C values in soil depend mainly on the δ^{13}C value of the plants contributing to SOM, which resembles a natural labelling of SOM (Balesdent *et al*., 1987). Plant δ^{13}C values depend on multiple factors, such as general discrimination during photosynthesis and biosynthesis of individual plant species that causes large isotopic differences between plant species, whereas other factors such as water stress, atmospheric CO_2 concentration, salinity, nitrogen availability and temperature lead to variation in isotopic values (Dawson *et al*., 2002). Environmental factors such as drought and subsequent water stress typically lead to ^{13}C enrichment in plant biomass from ^{13}C enrichment resulting from stomata closure. Consequently, there is less CO_2 uptake and utilization of assimilated ^{13}C to compensate for water loss (Fry, 2006). Subsequently, larger δ^{13}C can be expected in soil exposed to drier conditions. Similar to the distribution of TN and TC, in soil under C_3 plants a slight change in δ^{13}C can occur with soil depth, leading to ^{13}C enrichment of 2–3‰ with depth, which is related to incorporation of more root than litter-derived carbon and improved degradation of organic matter (Frank *et al*., 2011).

Soil moisture conditions have a strong effect on plant community, SOM content and stable isotopic composition, and so does topography. Generally drier conditions can be expected at the top of the slope where the effect of drainage decreases. Erosional losses and sediment transport of soil organic carbon can occur because of wetness, and slope gradient, curvature and direction (Bennie *et al*., 2006).

In general, drainage is thought to lead to a substantial reduction in TN and TC storage in soil, whereas evidence from long-term experiments on the importance of biodiversity, topography and spatial effects in this context is still missing. The Rowden Moor experiment, which was set up in southwest England at Rothamsted Research, North Wyke, UK, provides a unique opportunity to study such effects. In a long-term permanent grassland experiment, subsurface drainage was established at the depth of 30 cm in 1982, but for the plots of our study it was established in 1987. On the same site, undrained and drained meadows are available; the former can be used as a control because initially the grassland was the same on all plots. The following detailed hypotheses were examined:

1. *Plant community (by species diversity) in relation to topography and drainage*: (i) species diversity depends on topography because moisture steadily increases downslope and (ii) drained plots decrease species diversity.
2. *Soil chemistry in relation to sample depth, topography and drainage*: (i) TN, TC, the TC to TN ratio (TC/TN), δ^{15}N and δ^{13}C depend on soil depth, and TN, TC and TC/TN decrease down the soil profile, whereas δ^{15}N and δ^{13}C increase, (ii) TN, TC, TC/TN, δ^{15}N and δ^{13}C depend on topography and decrease downslope, (iii) enhanced mineralization of TN and TC contents decreases with drainage, (iv) TC/TN changes with drainage and (v) δ^{15}N and δ^{13}C values increase with drier soil conditions (i.e. in drained plots). Investigation of TC/TN provides context to the results of this study with respect to TN and TC individually.

These hypotheses were tested empirically by six separate linear mixed model (LMM) analyses (e.g. Piepho *et al*., 2003) using detailed information on biodiversity, where the percentage of vegetation cover of 120 sampling points (60 in each of the undrained and drained plots) was determined. At these points, soil samples were also taken at four depths and examined for their TN and TC contents, and for the natural abundance of N and C stable isotopes (δ^{15}N and δ^{13}C). Topographic information was available at all 120 sites in the form of elevation, slope, aspect and compound topographic index (CTI). Spatial autocorrelation in the data was also considered in the LMMs.

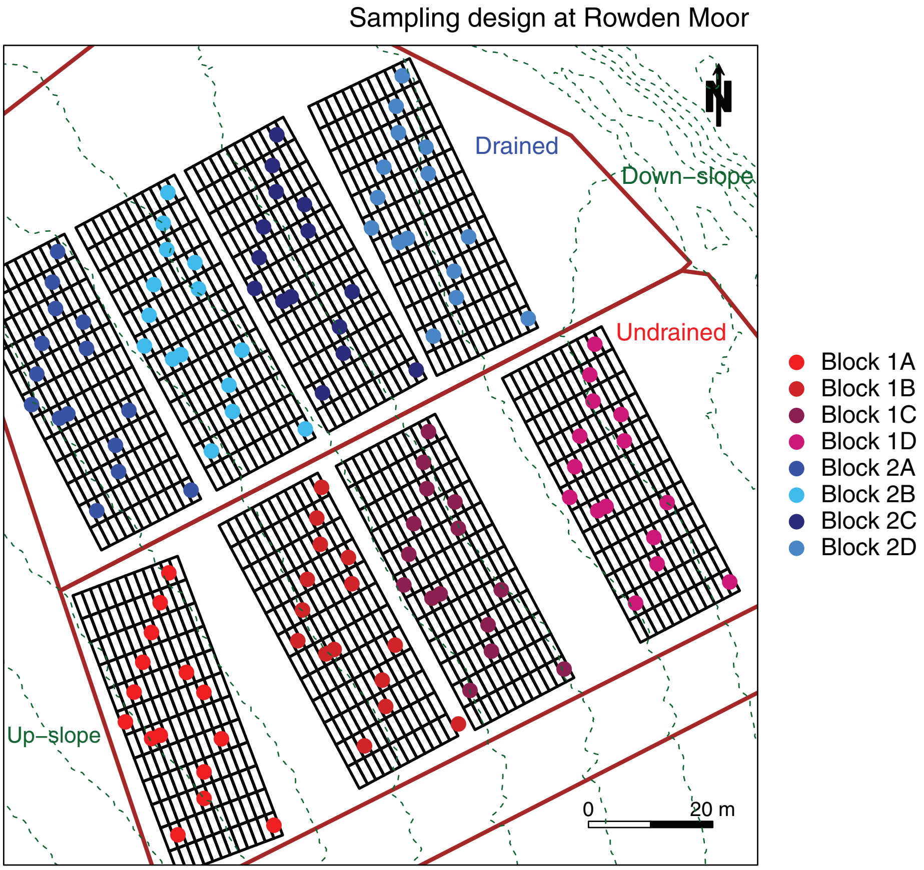


Figure 1 Sampling design at Rowden Moor. On both undrained (labelled 1) and drained (labelled 2) plots, four blocks (of size 50 m × 20 m) were allocated in a downslope direction. A rectangular 12 by 12 cell layout was overlaid on each block and the same random configuration of 15 sampling points was used in each of the four undrained and drained blocks (labelled A to D). Thus there are 120 sample sites in total. The study site is shown with elevation contours.

Material and methods

Site description

The experimental Rowden Moor site (50°45′N, 4°53′W) is near North Wyke in Devon, UK, about 7 km north of Dartmoor. The soil is classified as a Stagnic Vertic Cambisol (IUSS Working Group WRB, 2006) with a dense clay layer 30 cm below the surface (Harrod & Hogan, 2008). High precipitation in the winter and most summers and large clay contents result in large soil moisture content, and consequently periods of water logging of the soil (Harrod & Hogan, 2008). The annual mean temperature is 10.5 °C and the annual mean precipitation is 1056 mm, with most rainfall in the early winter months.

The experimental site is gently sloping (about 2–5°) from west to east. On the upper and bottom parts of the slope the relief is shallow. Experimental undrained plots (blocks labelled 1, undrained) and drained plots (blocks labelled 2, drained and hydrologically isolated since 1987) have not been fertilized with N mineral fertilizers since 1987 (Figure 1). Floristic composition in the early years of the experiment contained the following species: *Agrostis canina* L., *Agrostis stolonifera* L., *Anthoxanthum odoratum* L., *Cardamine pratensis* L., *Cerastium fontanum* Baumag, *Cynosurus cristatus* L., *Holcus lanatus* L., *Lolium perenne* L., *Phleum bertolonii* DC, *Phleum pratense* L., *Poa annua* L., *Poa trivialis* L., *Ranunculus repens* L., *Trifolium repens* L. and *Brachythecium* sp. There is no physical separation between the four blocks in the undrained plots or the four blocks in the drained plots, but the undrained and drained are separated. Immediately prior to this study, the pasture was grazed by cattle (~5 steers per hectare and ~6 months old) from May until October 2009, and was cut twice a year.

Six lysimeters are drained to 85 cm (well into the clay layer) by pipe drains at 40-m intervals across the slope, overlain by mole drains at 2-m spacing and at a shallower depth of 55 cm down the slope. Hydrological data show that for the drained plots, the average discharge by surface lateral and drainage pathways was 28% (with a standard deviation (SD) of 10.5%) and 72% (SD, 10.5%), respectively. The mole drains were renewed during 2010 (prior to this study) because they are more vulnerable to collapse than the deeper, more protected pipe drains. The improvement in drainage from re-moling was reflected in the comparison between 2010 and 2009 data, with average discharge of 46% (SD, 10.7%) and 54% (SD, 10.7%), respectively.

Sampling design

To mitigate natural spatial dependence within the plant communities and soil data, Latin square sampling + 3 (LSS + 3) for a rectangular 12 by 12 cell layout with LSS = 12 was applied (Dixon *et al*., 2010) to each block (of dimension 50 m by 20 m) in the undrained and drained plots (Figure 1). To determine broad topographic effects on plant and soil composition, four blocks were allocated in the downslope direction in the undrained and four in the drained plots. Thus, there were 15 cell sampling points for each block and a total of 120 sampling points. All sampling points were identified in the field by GPS.

Plant species and soil sampling

To identify changes in species composition of the plant community between drained and undrained plots, percentage vegetation cover of the plant species was determined at the 120 sites in early June 2011 using a 50 cm by 50 cm steel frame. At each observation point, these data were also summarized with Shannon's species diversity index (e.g. Hill, 1973).

For sampling soil profiles, a soil corer (2.5-cm diameter, 30-cm length) was used. Soil cores were divided into four depth intervals: 0–2.5, 2.5–7.5, 7.5–15 and 15–30 cm. Soil samples were stored in a freezer until sample preparation. Roots were picked out of the soil, and after drying the soil samples at 40 °C they were sieved to 2 mm and the fraction >2 mm was discarded. All samples were milled in a ball mill to a fine powder. Soil sampling was undertaken in January 2010. It is assumed that the different times of plant cover determination and soil sampling are of no consequence.

Elemental analysis

The TN and TC contents and δ^{15}N and δ^{13}C isotope composition were determined for all soil samples. The TN and TC were obtained by a Carlo Erba NA2000 analyser (CE Instruments, Wigan, UK). The δ^{15}N and δ^{13}C analyses were carried out with a SerCon 20–22 isotope ratio mass spectrometer (SerCon Ltd, Crewe, UK). Wheat flour (% C, 41.81; % N, 1.91; δ^{13}C, −26.41; δ^{15}N, 4.80) calibrated against IAEA-N-1 by Iso-Analytical, Crewe, UK, was used as a reference standard. The abundance ratios (R) of ^{15}N/^{14}N and ^{13}C/^{12}C are expressed in δ values per mill (‰) and are relative to the international VPDB (Vienna-Pee Dee Belemnite) and AIR (atmospheric N) standard, respectively (Dixon *et al.*, 2010):

$$\delta^{15}\mathrm{N} = \left[\frac{\left(R_{\mathrm{sample}} - R_{\mathrm{AIR}}\right)}{R_{\mathrm{AIR}}}\right] \cdot 1000,$$

$$\delta^{13}\mathrm{C} = \left[\frac{\left(R_{\mathrm{sample}} - R_{\mathrm{VPDB}}\right)}{R_{\mathrm{VPDB}}}\right] \cdot 1000.$$

Topographic data

At each sampling point, and throughout the sampling area, elevation, slope and aspect information were available from a 1-m resolution digital elevation model (DEM). The DEM data were also used to calculate the compound topographic index (CTI), a steady-state wetness index (Gessler *et al.*, 1995). All such DEM-derived data provide useful contextual information to the plant species and soil data, and provide a detailed alternative to block position. Elevation data ranged from a minimum of 142.7 m to a maximum of 149.6 m, slope from a minimum of 1.36° to a maximum of 6.07°, aspect from a minimum of 25.77° to a maximum of 99.69° and CTI from a minimum of 1.94 to a maximum of 6.95 (CTI is unitless).

Statistical methods

Unfortunately, the LSS sampling design is problematic in relation to the requirements for the most efficient statistical analysis. There is, in effect, just one replicate of each drainage treatment. Regardless of how many additional samples have been collected within each set of blocks, there is no true replication, only pseudo replication. It is still possible to work with these data, but because of the lack of replication one cannot be absolutely sure that what appears to be drainage effects are not just differences between the two areas that one might have seen if both had received the same drainage treatment.

The design at the block level does not provide 15 × 2 independent replicates of each block position because the sampling is undertaken within each block. The design means that each row and column of the grid is sampled once and only once before the three additional points are added. Given this, the design clearly does not provide observations that can be treated as independent. Consequently, there is no model for which the observations are conditionally independent either, which would have been the case if a stratified approach had been taken within the blocks with at least two samples per stratum. A further difficulty is that the same randomization was used in each block; therefore, even if the design had any desirable properties they would be obviated by the failure to randomize independently. Given these design issues, many standard statistical analyses and tests do not hold (e.g. ANOVA, Kruskal–Wallis H-tests, etc.) and as such, it was considered prudent to follow an LMM framework, as described below.

First-stage analyses: exploratory visualization. In the first instance, the plant species and soil data were summarized using conditional histograms and boxplots, where the conditioning relates to: (i) drainage and (ii) ordered block position. Ordered block position provides a broad topographic measure because blocks A to D slope downwards (Figure 1). For the soil data only, conditional histograms are also given for the four depths. Conditional histograms and boxplots provide a useful way to assess the study's hypotheses. The plant species and soil data were also summarized visually by bar plots and maps. Further exploration of the plant species data is provided through a series of hierarchical cluster analyses (Ward's method with a Bray–Curtis distance measure; see Murtagh & Legendre, 2014) applied to the percentage vegetation cover. This might provide greater insight into this rich dataset than the summary form with Shannon's species diversity index.

Second-stage analyses: linear mixed models. To test the study's hypotheses formally, a series of six LMMs were fitted with GenStat 18 (VSN International, 2015) for each of the following response variables: species diversity, TN, TC, TC/TN, δ^{15}N and δ^{13}C. Where

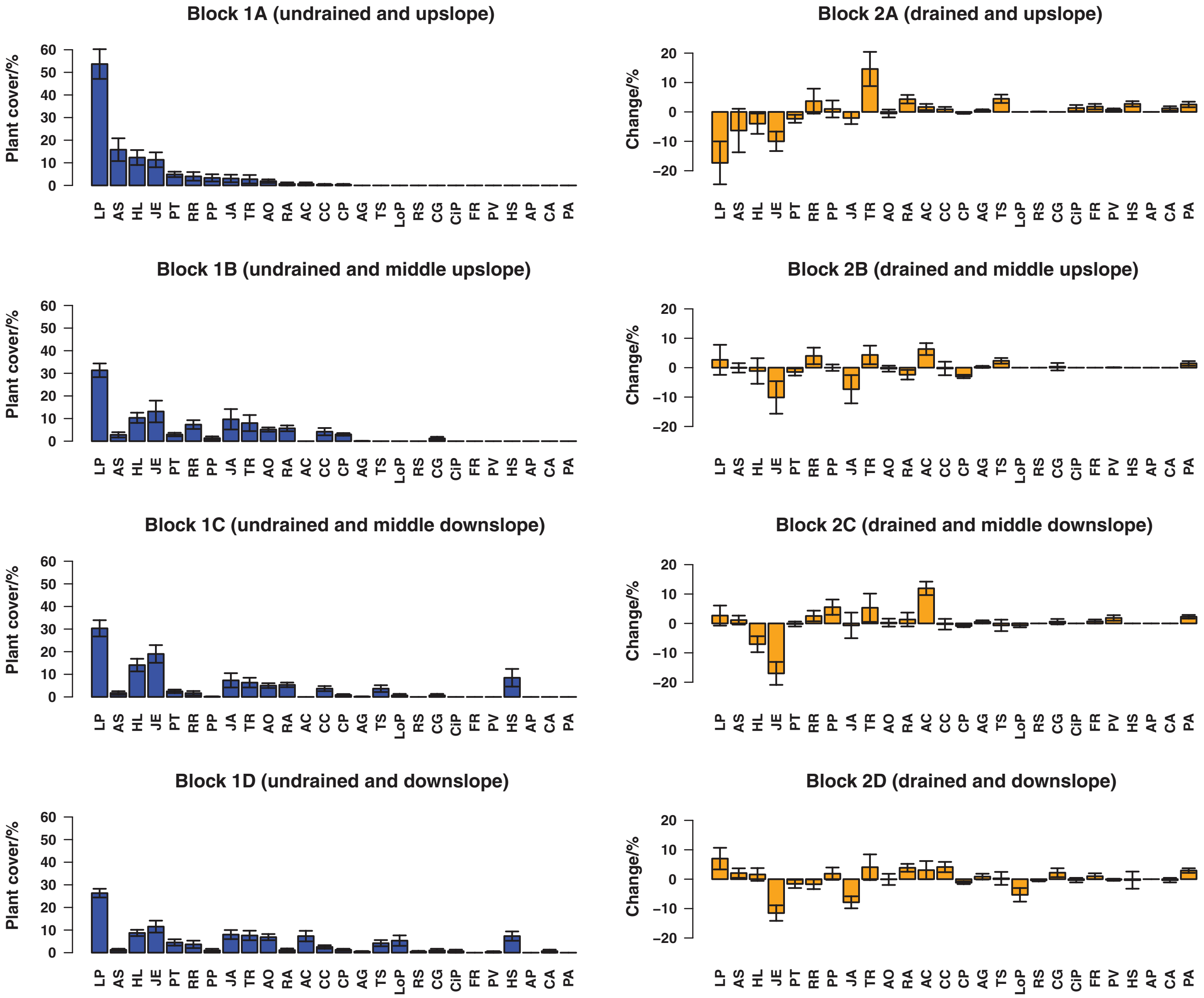


Figure 2 Percentage plant cover (blue bars) of the undrained blocks and the change in plant cover related to the drained blocks at the same slope position (orange bars). For the order of plant species, Block 1A was chosen as reference. The plant species are: *Lolium perenne* (LP), *Agrostis stolonifera* (AS), *Holcus lanatus* (HL), *Juncus effuses* (JE), *Poa trivialis* (PT), *Ranunculus repens* (RR), *Phleum pratense* (PP), *Juncus acutiflorus* (JA), *Trifolium repens* (TR), *Anthoxanthum odoratum* (AO), *Ranunculus acris* (RA), *Agrostis capillaris* (AC), *Cynosurus cristatus* (CC), *Cardamine pratensis* (CP), *Alopecurus geniculatus* (AG), *Taraxacum spec* (TS), *Lotus pedunculatus* (LoP), *Ranunculus spec* (RS), *Cerastium glomerate* (CG), *Cirsium palustre* (CiP), *Festuca rubra* (FR), *Purella vulgaris* (PV), *Hypericum spec* (HS), *Alopecurus pratensis* (AP), *Cirsium arvense* (CA) and *Poa annua* (PA).

appropriate, transformations of these response variables were considered based on residual plots. Here TN, TC and δ^{15}N all required a square-root transformation to satisfy the LMM assumptions (δ^{15}N also required an offset of 33 to make all values positive). Initially, a full factorial structure of *fixed terms* was considered for all LMMs; that is, including the main effects and all interactions of drainage, elevation, slope, aspect, CTI and depth (except for the species diversity response where depth was not applicable). Drainage was a two-level factor, depth was a four-level factor, whereas all other explanatory variables are continuous covariates.

The initial random structure considered for species diversity was block|sample-location (where block was an eight-level factor and sample-location was a 15-level factor), while accounting for spatial autocorrelation between sample points with a power variogram model based on Euclidean distance between points. The initial random structure for all other responses (TN, TC, TC/TN, δ^{15}N and δ^{13}C) was block|sample-location|depth (where depth was a four-level factor), while accounting for spatial correlation between sample points with a power variogram model, and accounting for correlation between depths at the same point with an auto-regressive order 1 (AR-1) model. Simplification of the random model was considered by comparing the deviances of the models with and without spatial terms. Simplification of the fixed model was considered by backward selection, starting with the full model (all interactions included) and removing the least significant term until no more could be removed.

Results

Plant community in relation to topography and drainage (H1)

The change in percentage vegetation cover across the undrained and drained plots is depicted in Figure 2. Results suggest that at the Rowden Moor field experimental site after 24 years of drainage, a shift in plant community assemblage occurred on this clay soil from a *Lolium perenne*-dominated grassland with patches of *Juncus* species, towards a grassland community composed of *Lolium perenne* and *Trifolium repens*. Species that appeared to suffer the most from the drainage effect included: *Lolium perenne* (on the upper slopes only), *Juncus effuses* L. and *Juncus acutiflorus* Ehrh. Ex Hoffm. Species that appeared to benefit the most from the drainage included: *Trifolium repens, Agrostis capillaris* L., *Ranunculus repens* and *Poa annua*.

Output from hierarchical clustering of the percentage vegetation cover data is presented in Figure 3 and in Figure 4(a). In the first instance, four separate hierarchical cluster analyses were applied to the percentage data in paired blocks 1A and 2A, 1B and 2B, 1C and 2C, and 1D and 2D (see also Figure 1). The four dendrograms in Figure 3 show some evidence that the drained and undrained plots contain different plant communities, but the strength of this evidence depends on the paired block position. This is especially true for blocks 1D and 2D at the bottom of the slope where the clustering outputs demark two plant communities by the block from which they were sampled. Second, a single hierarchical cluster analysis was applied to the full percentage vegetation cover dataset, and the dendrogram was cut to form two, four and eight groups. The results are presented as group maps in Figure 4(a); the two-group map appears to have some limited success in demarking two plant communities by drainage. The four- and eight-group maps are not promising because species composition does not appear to depend on drainage or block position.

Conditional distributions for plant species diversity by drainage and block position, and by drainage only, are given in Figure 4(b,c), together with the species diversity map in Figure 4(d). These distributions are complemented by the LMM results given in Table 1. Clearly plant species diversity tends to be much stronger at the lower part of the slope, which would relate to the wettest conditions present (note that species diversity relates negatively and significantly to elevation ($P < 0.001$) from the LMM in Table 1). Species diversity does not depend on any other topographical variables, nor does it depend on whether the plots were drained or not (see LMM in Table 1). Thus, moisture (and drought) does not appear to have an effect in this respect. Although species diversity depended on elevation only as a topographic effect, a spatial element to this process was retained in the LMM (the only instance in this study). This might, in part, be because all other topographic effects (slope, aspect and CTI), which can act as ancillary spatial effects, were removed from the LMM as part of its fitting procedure.

Soil chemistry regarding depth, topography and drainage (H2)

Conditional distributions by drainage for soil chemistry at the four depths are given in Figure 5. Conditional distributions by drainage and block position for soil chemistry at the four depths are given in Figures 6 and 7. Maps for soil chemistry are given in Figures 8 and 9. These visual data summaries are placed in statistical context by the corresponding LMM results given in Tables 2–6.

From these analyses, soil composition clearly depends on the sampling depth for both undrained and drained plots. For TN, TC and TC/TN, values tend to decrease down the soil profile (Figures 5, 6 and 8), whereas for δ^{15}N and δ^{13}C, values tend to increase (Figures 5, 7 and 9). These results are statistically significant ($P < 0.001$ in all cases), as shown by the LMM results in Tables 2–6, where their directions are given by negative or positive effects. Various interaction terms between sampling depth, topography and drainage are included in the models for TN, TC and δ^{13}C. Of these, the following showed a statistically significant effect: TN with depth, aspect and drainage ($P = 0.050$); TN with depth, CTI and drainage ($P = 0.017$); TN with depth, aspect, CTI and drainage ($P = 0.002$); TN with depth, slope, elevation and drainage ($P = 0.041$); TN with depth, slope, aspect and drainage ($P = 0.017$); TC with depth, slope and drainage ($P = 0.027$); TC with depth, CTI and drainage ($P = 0.010$); TC with depth, slope, elevation and drainage ($P = 0.033$); TC with depth, CTI, slope, elevation and drainage ($P = 0.039$); TC with depth, CTI, aspect, slope and drainage ($P = 0.007$) and δ^{13}C with depth, CTI and drainage ($P = 0.002$). Similarly, interactions between sampling depth and topography were included for TC, TC/TN, δ^{13}C and δ^{15}N, where the following showed a significant effect: TC with depth and aspect ($P = 0.023$); TC with depth, aspect and slope ($P = 0.026$); TC/TN with depth and elevation ($P < 0.001$); δ^{13}C with depth and elevation ($P < 0.001$); δ^{13}C with depth, aspect and slope ($P = 0.016$); δ^{13}C with depth, CTI, slope and elevation ($P = 0.045$); δ^{15}N with depth and elevation ($P < 0.001$) and δ^{15}N with depth, CTI and slope ($P < 0.001$). There was a significant interactive effect between sampling depth and drainage for TN only ($P = 0.050$).

In general, TN and TC depend strongly on topographic characteristics with a decrease downslope (see Figures 6 and 8, noting the significant positive relations for TN with elevation ($P = 0.010$) and with CTI ($P = 0.025$); and for TC with elevation ($P = 0.022$) and with CTI ($P = 0.022$) from the LMMs in Tables 2 and 3), whereas for TC this relation interacts with sampling depth. The TC/TN ratio also depends on topography, with significant positive relations with elevation and with CTI ($P = 0.047$ and $P = 0.032$, respectively; see the LMM in Table 4), suggesting a decrease downslope. However, compared with TN and TC, topographic and soil-depth relations with TC/TN are relatively simple with far fewer interaction terms (see Table 4 and Figure 8). The δ^{15}N and δ^{13}C values again tend to decrease downslope (see Tables 5 and 6, and Figures 7 and 9). There is a significant relation to the interaction of δ^{15}N with slope and CTI ($P = 0.037$), to the interaction between δ^{15}N and elevation, aspect and CTI ($P = 0.035$) and to the interaction between elevation, slope and CTI ($P = 0.044$), whereas δ^{13}C relates significantly to elevation

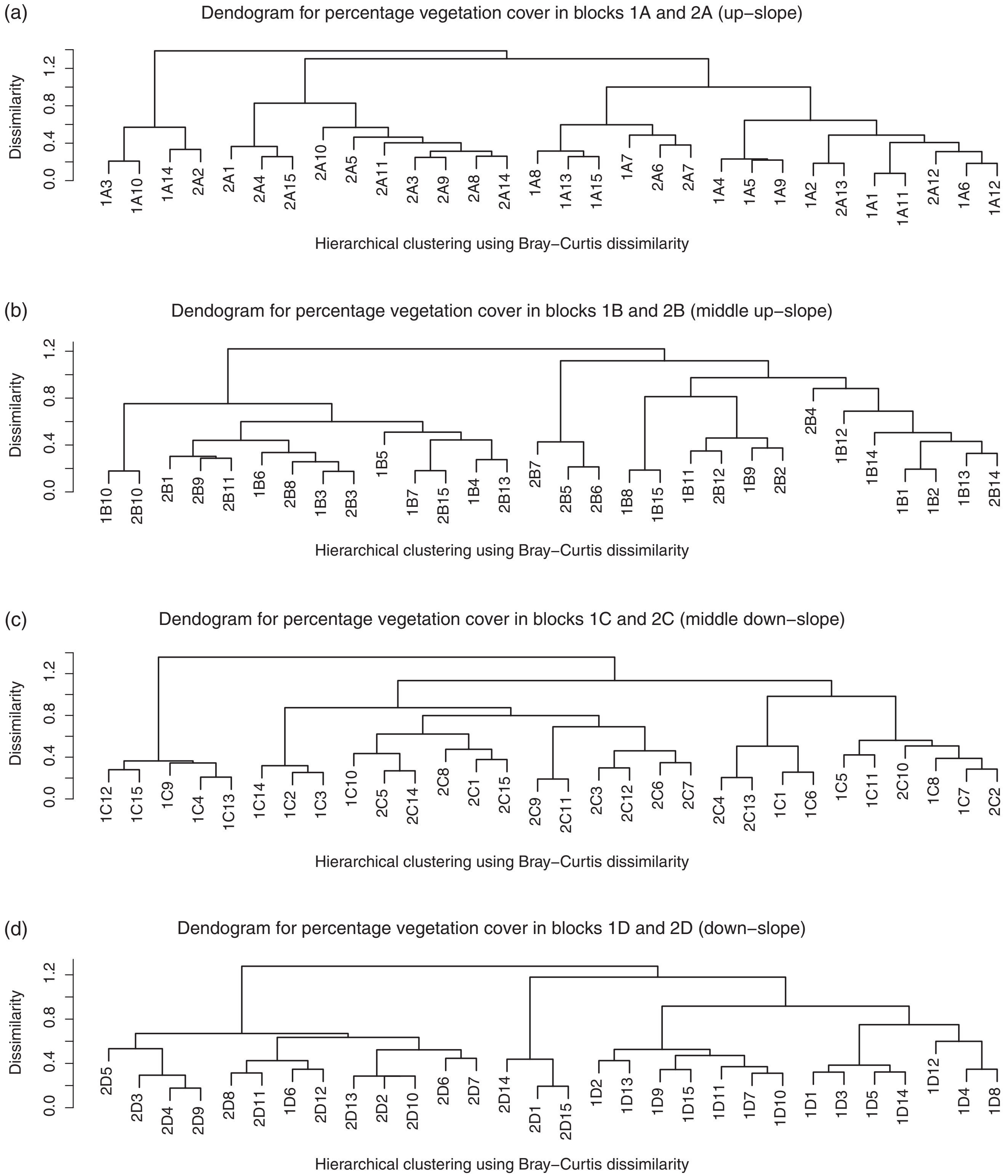
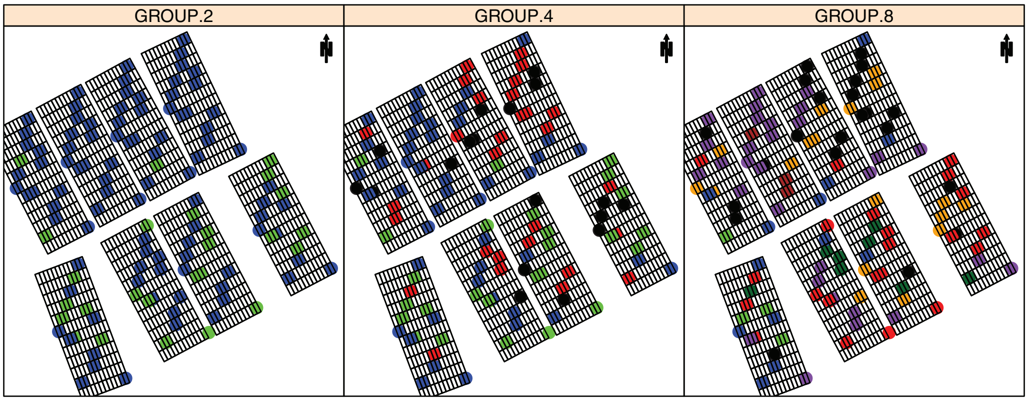


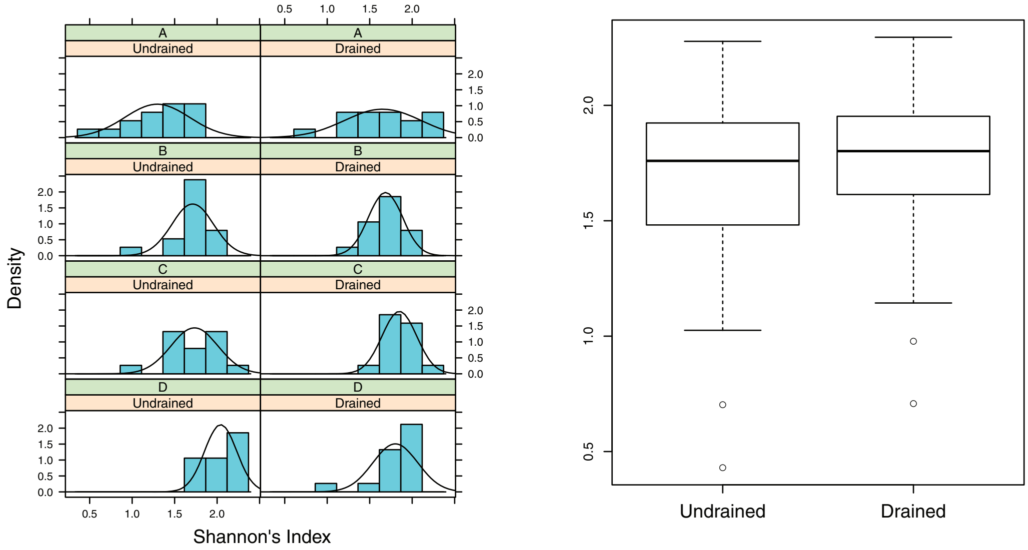
Figure 3 Dendograms from four hierarchical cluster analyses (with Bray−Curtis dissimilarity) applied to the percentage vegetation cover in (a) paired blocks 1A and 2A, (b) paired blocks 1B and 2B, (c) paired blocks 1C and 2C and (d) paired blocks 1D and 2D.

(a) Hierarchical clustering results for percentage vegetation cover with two, four and eight dendogram cuts



(b) Conditional histograms for species diversity

(c) Conditional boxplots for species diversity



(d) Species diversity (Shannon's Index)

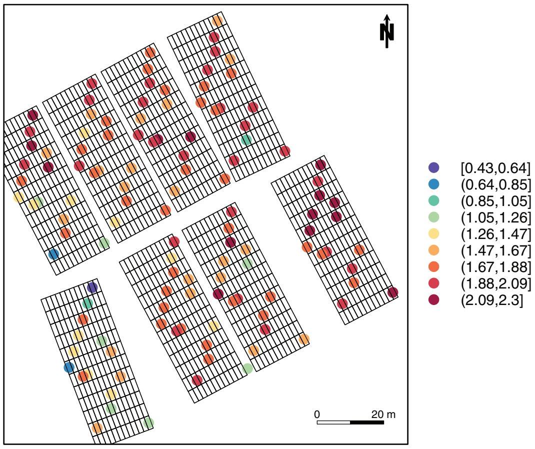


Figure 4 (a) Maps of results from hierarchical clustering for percentage vegetation cover at all sites (note the colour coding has no meaning apart from demarking the groupings and does not transfer between the two, four and eight groupings). Conditional distributions for species diversity (Shannon's Index) by (b) drainage and block position (downslope of blocks runs from A to D) and (c) drainage only. Map of species diversity (d).

Table 1 Linear mixed model for Shannon's Index (response variate)

REML variance components analysis (final model)					
Response variate	Fixed model	Random model	Number of units	–	–
Shannon's Index	Constant + Elevation*	Block + Block. sample–location	120	–	–
Covariance structures defined for random model:					
Term	Factor	Model	Order	Number of rows	–
Block. sample–location	Block	Identity	1	8	–
	Sample–location	Power function	1	15	–
Estimated variance components:					
Random term	Component	Standard error	–	–	–
Block	−0.00196	0.00565	–	–	–
Residual variance model:					
Term	Factor	Model (order)	Parameter	Estimate	Standard error
Block. sample–location	–	–	Residual variance	0.0912	0.0140
	Block	Identity	–	–	–
	Sample–location	Power (1)	Phi-1	0.7493	0.0707
Deviance and degrees of freedom:					
−165.42	115	–	–	–	–
Tests for fixed effects: sequentially adding to fixed model or dropping individual terms from full fixed model:					
Fixed term	Wald statistic	Numerator degrees of freedom	F-statistic	Denominator degrees of freedom	P
Elevation	36.90	1	36.90	7.6	< 0.001
Table of effects for constant (estimate and standard error):					
1.708	0.031	–	–	–	–
Table of effects for elevation (estimate and standard error):					
−0.106	0.018	–	–	–	–

*Significant fixed effect ($P < 0.05$).
All covariates centered. Estimated covariance models not reported.

($P = 0.038$). Topographic information also interacts with soil depth, for δ^{15}N and δ^{13}C values. Interestingly, although TN, TC, TC/TN, δ^{15}N and δ^{13}C each have clear spatial trends (Figures 8 and 9), topographic data alone can resolve this because all the LMMs drop their spatial term as part of the fitting procedure.

The plots and maps in Figures 5, 6 and 8 suggest that distributions of TN and TC clearly differ between the undrained and drained plots, where the magnitude of such differences relates to sample depth and topography. In general, TN and TC appear to decrease in the drained plot. These visual interpretations are endorsed by the LMM results in Tables 2 and 3 because drainage is included in the models, and TN and TC values tend to be larger in the undrained plot (i.e. positive effects in LMM results tables). However, drainage in isolation is not a significant effect for TN and TC (with $P = 0.188$ and $P = 0.210$, respectively) because the LMM results confirm significant interactions only between various combinations of drainage, sample depth and topography for the distributions of TN and TC (see above). Furthermore, the TC/TN ratio does not depend on drainage in isolation or in any interactive sense (Table 4). For the isotopes, there is also no evidence that δ^{15}N depends on drainage (Table 5), although there are differences in Figures 5 and 9. Conversely, δ^{13}C depends significantly on drainage ($P = 0.041$, see Table 6 and Figures 5 and 9) and tends to be depleted (decrease) in the drained plots (i.e. a positive effect in the LMM results table). There are also significant interaction effects between topography and drainage for δ^{13}C (i.e. for aspect interacting with drainage ($P = 0.020$), and for interactions between elevation, aspect and drainage ($P = 0.003$)).

Discussion

Plant species composition

It is somewhat surprising that the effect of the long-term drainage on moisture (and drought) did not influence species diversity because it is well known that management factors such as drainage can have a large effect on grassland composition. Furthermore, Grootjans *et al.* (2005) have reported a much clearer trend for fen meadows. However, Jentsch *et al.* (2011) did not observe changes in biodiversity, but found that plant communities were more stable when exposed to annual drought.

Plant species tended to be more diverse at the lower part of the slope (i.e. where the wettest soil conditions were present in both the drained and undrained plots). This can be related to the fact that there are plateaus at the top and bottom of the slope; these are within the overall imposed hydrological isolation of each 1-ha field lysimeter from the meadows situated above and around it. Furthermore, the lower part of the slope is the nearest to a hedge, is more sheltered and might have more seed deposited from the non-grazed part of the field outside a wire fence.

The smallest changes in plant community were observed at the bottom of the slope where the level patches on the drained and undrained plots might have led to rather similar conditions. In

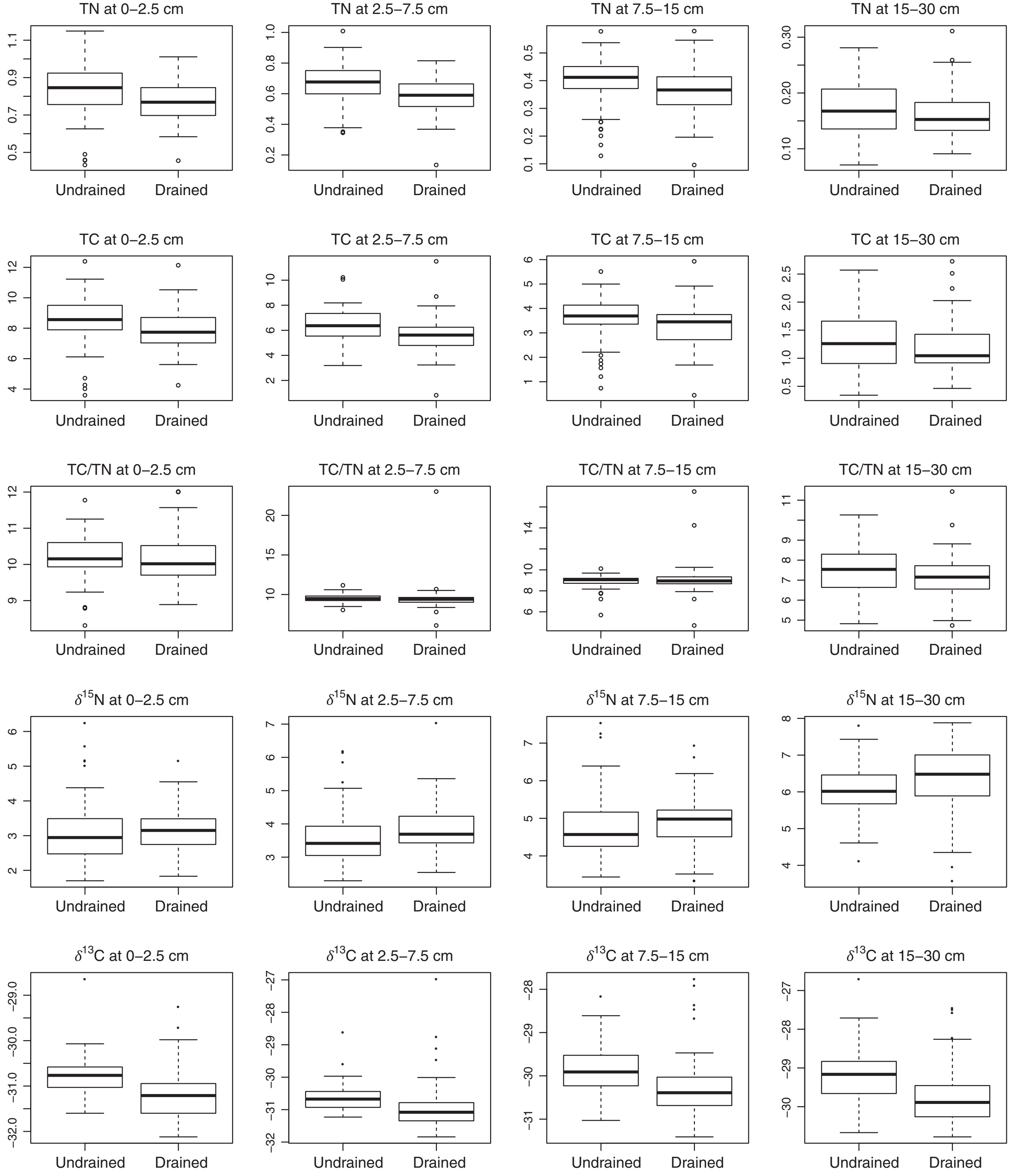


Figure 5 Conditional boxplots by drainage for soil total nitrogen (TN), soil total carbon (TC), TC/TN and stable isotopic (δ^{15}N, δ^{13}C) contents at the four soil depths.

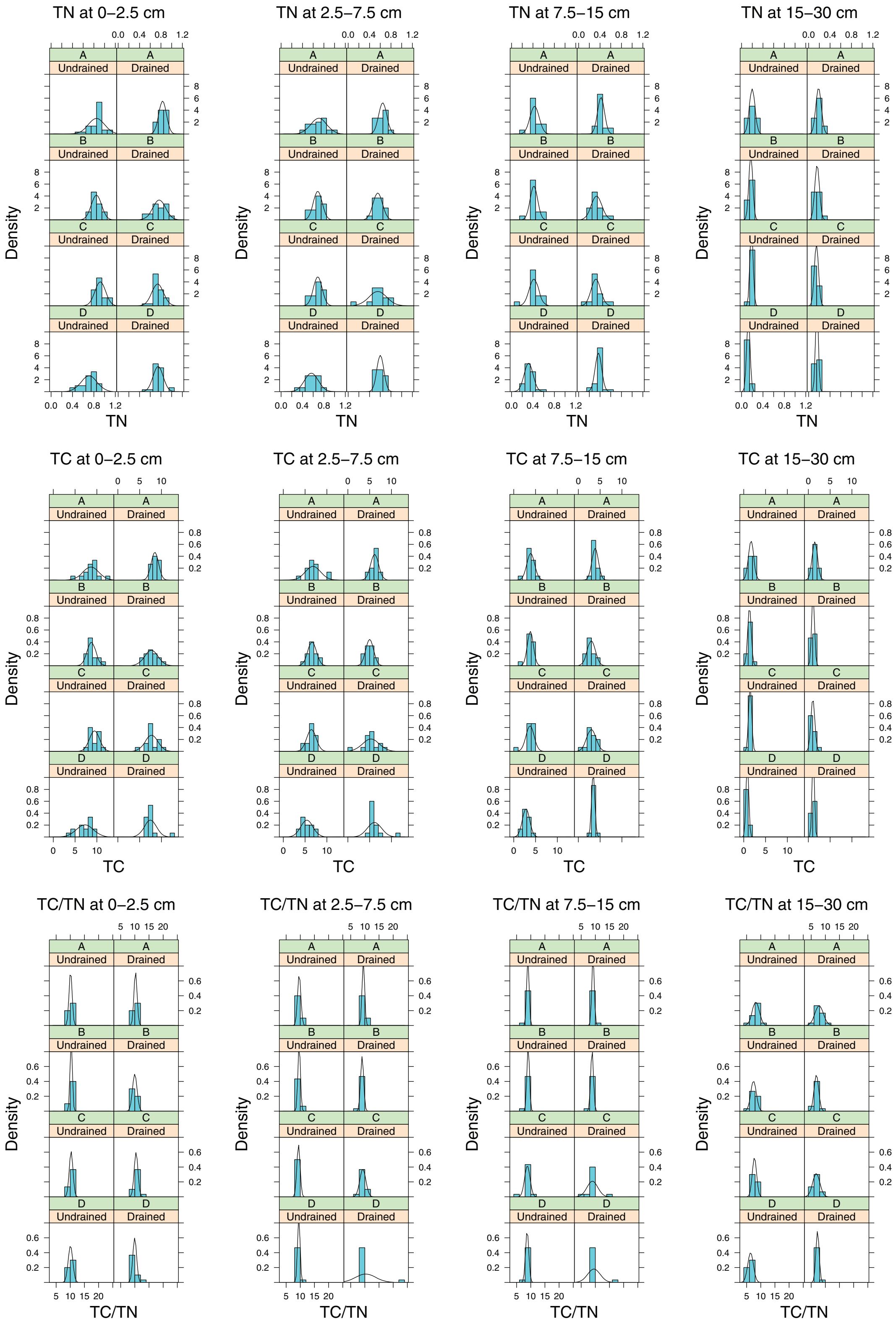


Figure 6 Conditional distributions by drainage and block position for soil total nitrogen (TN), soil total carbon (TC) and TC/TN at four different soil depths. Downslope of blocks from A to D.

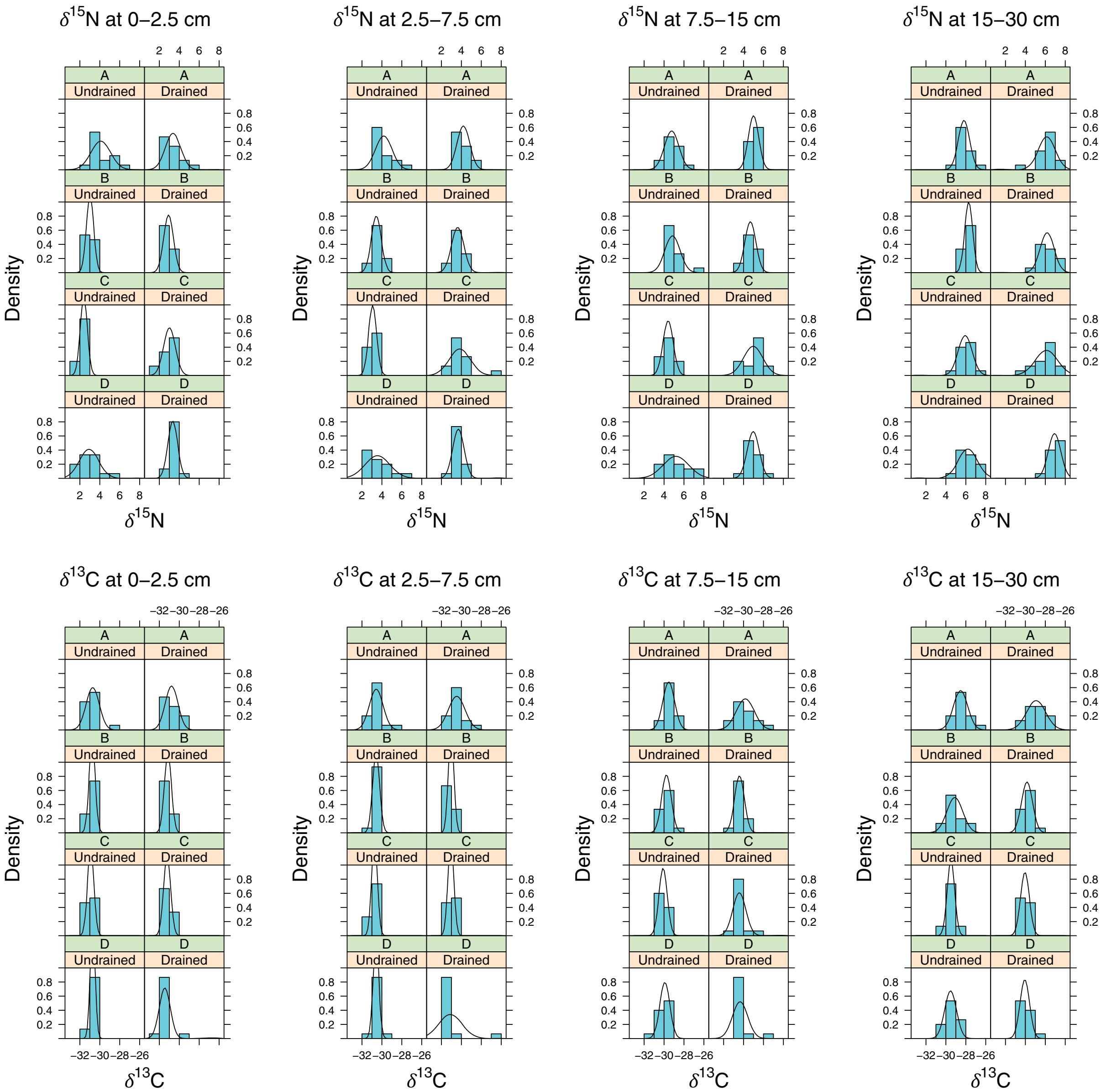
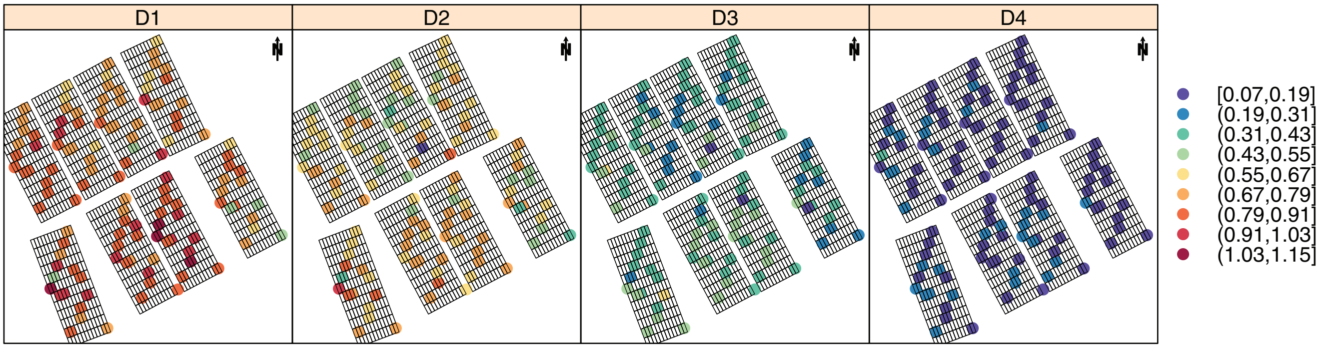


Figure 7 Conditional distributions by drainage and block position for stable isotopic (δ^{15}N, δ^{13}C) contents at four different soil depths. Downslope of blocks from A to D.

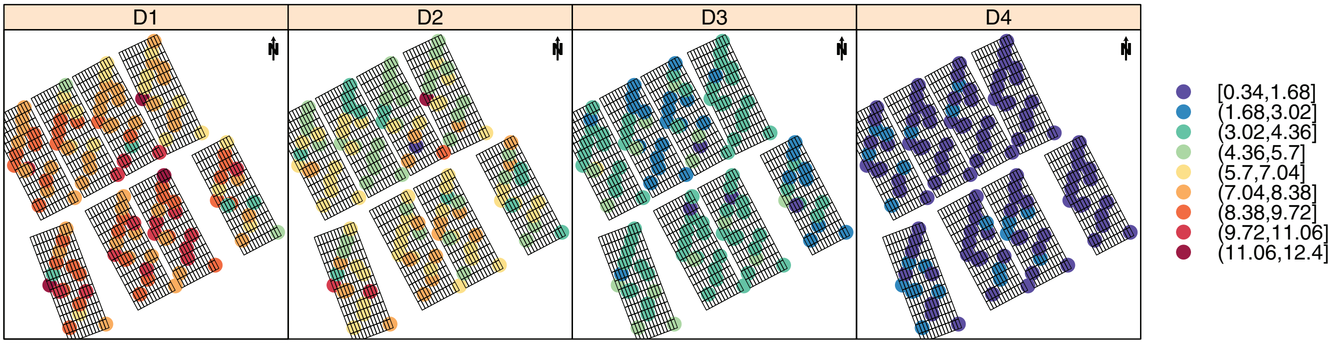
contrast, on the lower slope, the drainage might have strongly disturbed water storage and water flow, leading to very different hydrological conditions between the drained and undrained plots. Change of plant community and species richness with elevation has been reported previously (e.g. Bennie *et al*., 2006). The limitation of plant-available water was described to be the most important reason for the decrease in plant biodiversity.

Although not formally tested, overall our study suggested that the drier conditions in the drained plot shifted the plant species composition from a *Lolium perenne*-dominated grassland with widely distributed patches of *Juncus* species towards a grassland community composed of *Lolium perenne* and *Trifolium repens*. The shift included a decrease in Juncaceae, which are adapted to large soil moisture contents, and an increase in dicotyledons such as legumes and herbs, which are more competitive species and can endure possible mid-summer soil moisture deficits (Grootjans *et al*., 2005). At Rowden Moor the soil is clayey with a large water storage capacity, which might have led to the change in plant community composition, but not the overall diversity because all plants are already adapted to the soil type and the moist site conditions. Seasonal drought, however, probably reduced the competitiveness of the rushes at the site, and where they could not resist some drought periods during the past, legumes and other plants filled the gaps where the rushes had disappeared. Our study suggests that

(a) Spatial distribution of TN at four depths (shallow to deep)



(b) Spatial distribution of TC at four depths (shallow to deep)



(c) Spatial distribution of TC/TN at four depths (shallow to deep)

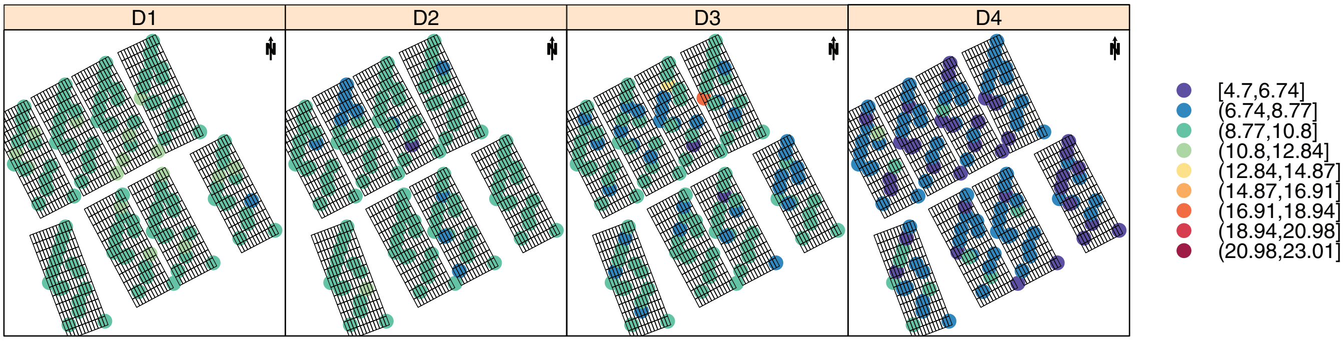


Figure 8 Spatial distributions for (a) soil total nitrogen (TN), (b) soil total carbon (TC) and (c) TC/TN at the four different soil depths: 0–2.5 (D1), 2.5–7.5 (D2), 7.5–15 (D3) and 15–30 cm (D4).

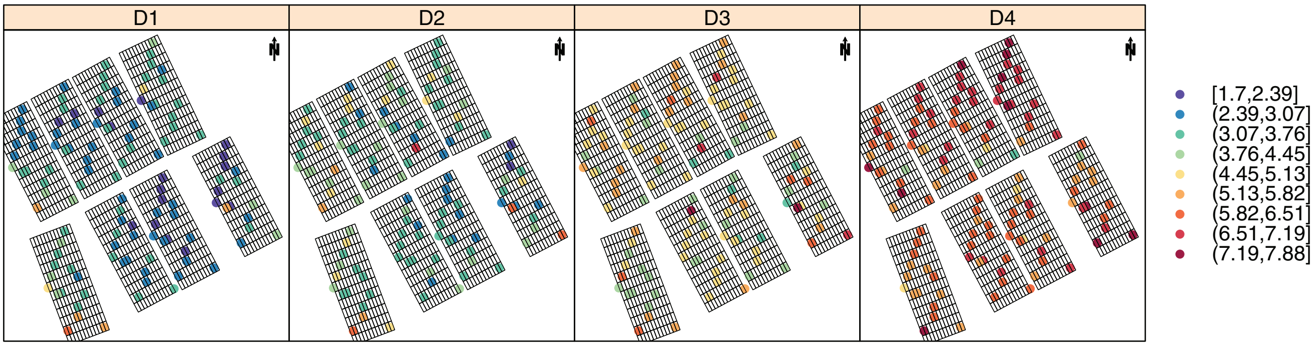
moist meadows under artificially imposed drainage conditions do not necessarily develop towards more typical grasslands associated with a loss in biodiversity.

Elemental soil composition: TN, TC and TC/TN

The observed decrease in TN, TC and TC/TN values with depth is generally observed in soil (Jobbágy & Jackson, 2001), including permanent grasslands (e.g. Gregory *et al.*, 2016). The decrease in TN and TC contents with soil depth results from declining input of above-ground litter with depth and its decomposition (Chen *et al.*, 2009).

Both TN and TC depended significantly on drainage, but this interacted with topographic effects and depth. Drainage in isolation was not a significant effect. The relation between TN and TC with elevation might result from the significant changes in plant community (i.e. diversity) observed, mainly associated with changes to the input of organic matter. An increase in soil N contents could be expected in the drained soil of Rowden Moor because of the relative increase in legumes and thus symbiotic N_2-fixing bacteria in the soil associated with *Trifolium repens* (Carlsson & Huss-Danell, 2003). However, this was not observed because TN decreased throughout the drained plot. This might be related to fast mineralization of N in the soil or direct assimilation of N, leaching of N or its release by

(a) Spatial distribution of δ^{15}N at four depths (shallow to deep)



(b) Spatial distribution of δ^{13}C at four depths (shallow to deep)

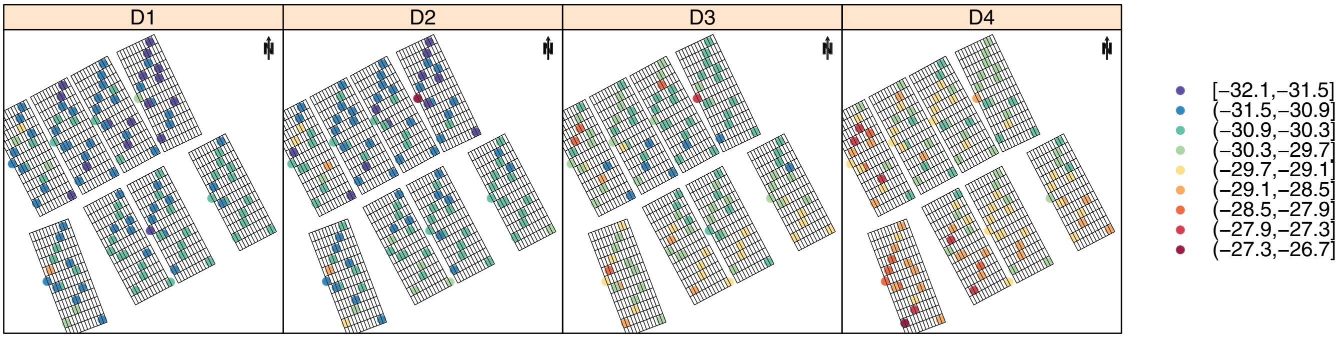


Figure 9 Spatial distributions for (a) stable isotopic (δ^{15}N) and (b) stable isotopic (δ^{13}C) at the four different soil depths: 0–2.5 (D1), 2.5–7.5 (D2), 7.5–15 (D3) and 15–30 cm (D4).

denitrification (Bedard-Haughn *et al*., 2003). Direct assimilation of N was observed by Grime *et al*. (2008), who stated that N fixation by *Trifolium repens* often encourages the temporary dominance of grasses, which can lead to a reduced N status of the soil. Legumes probably do not contribute significantly to the total N fixation at Rowden Moor, where N contents were large compared with other soils (Chen *et al*., 2009).

The significant effects of drainage and topography on TN and TC, especially from the upper three soil-sample depths, could be related to differential changes in the groundwater table and outer membrane translocation between the drained and undrained plots. The groundwater table is supposed to increase downslope on these Rowden Moor fields because they border an adjacent stream. This also leads to enhanced stagnic properties of the soil, even at shallower depths, especially at the bottom of the slope. This higher groundwater table may reduce the rate of decomposition of organic matter and thereby enhance soil N and C contents in the undrained plots, which has also been observed in other studies (e.g. Abid & Lal, 2008).

The significant relations between TC/TN and topography and soil depth were relatively simple compared with that found for TN and TC. This result probably indicates that any effect of soil moisture conditions modified both TN and TC similarly; therefore, the TC/TN ratio will not change as observed previously (Abid & Lal, 2008).

Elemental soil composition: δ^{15}N and δ^{13}C

For soil δ^{15}N, there was no statistical evidence for it to be larger in the drained plots, as a result of the combined effect of a shift towards N-fixing legumes in the plant community and less ^{15}N discrimination under drier conditions (Handley *et al*., 1999). Previous research has suggested that legumes in grassland communities do not necessarily lead to larger N contents or δ^{15}N values (Elgersma & Hassink, 1997). This is because incorporated N can be consumed easily by other plants and the relatively large N contents at Rowden Moor dilute the contribution of fixed N. Soil δ^{13}C showed significantly smaller values in the drained plots, which is the reverse to what was expected; δ^{13}C usually increases in plants when exposed to drier conditions because of reduced stomatal conductance and selective enrichment of ^{13}C (Robinson *et al*., 2000). This might be explained by the indirect shift in the plant community, and not the direct effect of the reduction in soil moisture status.

The insignificant effect of drainage on δ^{15}N values suggests that fractionation processes in the plant–soil system were not strongly affected by drainage, despite smaller soil moisture content and significant changes in TN content within the upper soil layers. Another possibility is that N incorporation by N_2-fixing bacteria of the increasing legume population and the drainage effect are in competition in relation to δ^{15}N values. Legumes are associated with small isotopic fractionation values during N_2 fixation, with values

Table 2 Linear mixed model for total nitrogen (TN) (response variate)

REML variance components analysis (final model):					
Response variate	Fixed model	Random model	Number of units	–	–
TN	Constant + Drained + Elevation* + Slope + Aspect + CTI* + Depth* + Drained.elevation + Drained. slope + Elevation.slope + Drained. aspect + Slope.aspect + Drained.CTI + Aspect.CTI + Drained.depth* + Elevation.depth + Slope.depth + Aspect.depth + CTI.depth + Drained.elevation.slope + Drained.slope.aspect + Drained.aspect.CTI + Drained.elevation.depth + Drained.slope.depth + Elevation.slope.depth + Drained.aspect.depth* + Slope.aspect.depth + Drained.CTI.depth* + Aspect.CTI.depth + Drained.elevation.slope.depth* + Drained.slope.aspect.depth* + Drained.aspect.CTI.depth*	Block + Block.sample–location + Block. sample–location.depth	480	–	–
Covariance structures defined for random model:					
Term	Factor	Model	Order	Number of rows	–
Block.sample-location.depth	Block	Identity	1	8	–
	Sample–location	Identity	0	15	
	Depth	Auto–regressive	1	4	–
Estimated variance components:					
Random term	Component	Standard error	–	–	–
Block	0.000396	0.000401	–	–	–
Block. sample-location	0.000162	0.001037	–	–	–
Residual variance model:					
Term	Factor	Model (order)	Parameter	Estimate	Standard error
Block.sample-location.depth	–	–	Residual variance	0.00375	0.00107
	Block	Identity	–	–	–
	Sample–location	Identity	–	–	–
	Depth	AR-1	Phi-1	0.6099	0.1126
Deviance and degrees of freedom:					
−1641.33	412	–	–	–	–
Table of effects for constant (estimate and standard error):					
0.8819	0.0135	–	–	–	–
Table of effects for drained (drained, undrained and standard error of differences):					
< 0.000001	0.02775	0.01908	–	–	–
Table of effects for elevation (estimate and standard error):					
0.01103	0.01085	–	–	–	–
Table of effects for slope (estimate and standard error):					
−0.000461	0.010315	–	–	–	–
Table of effects for aspect (estimate and standard error):					
−0.000406	0.000604	–	–	–	–
Table of effects for CTI (estimate and standard error):					
0.003497	0.009173	–	–	–	–
Table of effects for depth (0–2.5, 2.5–7.5, 7.5–15, 15–30 cm and average standard error of differences):					
< 0.0001	−0.1170	−0.2826	−0.4815	0.0092	–
Table of effects for drained.elevation (drained, undrained and standard error of differences):					
< 0.00001	0.00575	0.01283	–	–	–
Table of effects for drained.depth (0–2.5, 2.5–7.5, 7.5–15, 15–30 cm and average standard error of differences):					
Drained	< 0.00001	< 0.00001	< 0.00001	< 0.0000	–
Undrained	< 0.00001	0.00684	−0.00824	−0.02280	0.01338
Table of effects for elevation.depth (0–2.5, 2.5–7.5, 7.5–15, 15–30 cm and average standard error of differences):					
< 0.00001	−0.000141	0.000779	0.002924	0.008904	–
Table of effects for drained.elevation.depth (0–2.5, 2.5–7.5, 7.5–15, 15–30 cm and average standard error of differences):					
Drained	< 0.000001	< 0.000001	< 0.000001	< 0.000001	–
Undrained	< 0.000001	0.005393	0.000860	−0.000580	0.01053

*Significant fixed effect ($P < 0.05$).

All covariates centred. Not reported: estimated covariance models and tests for fixed effects. Partially reported: tables of effects.

AR-1, auto-regressive order 1 model. CTI, compound topographic index

Table 3 Linear mixed model for total carbon (TC) (response variate)

REML variance components analysis (final model):					
Response variate	Fixed model	Random model	Number of units	–	–
TC	Constant + Drained + Elevation* + Slope + Aspect + CTI* + Depth* + Drained.elevation + Drained.slope + Elevation.slope + Elevation.aspect + Slope.aspect + Drained.CTI + Elevation.CTI + Slope.CTI + Aspect.CTI + Drained.depth + Elevation.depth + Slope.depth + Aspect.depth* + CTI.depth + Drained.elevation.slope + Elevation.slope.aspect + Drained.elevation.CTI + Drained.slope.CTI + Elevation.slope.CTI + Elevation.aspect.CTI + Slope.aspect.CTI + Drained.elevation.depth + Drained.slope.depth* + Elevation.slope.depth + Elevation.aspect.depth + Slope.aspect.depth* + Drained.CTI.depth* + Elevation.CTI.depth + Slope.CTI.depth + Aspect.CTI.depth + Drained.elevation.slope.CTI + Elevation.slope.aspect.CTI + Drained.elevation.slope.depth* + Elevation.slope.aspect.depth + Drained.elevation.CTI.depth + Drained.slope.CTI.depth + Elevation.slope.CTI.depth + Elevation.aspect.CTI.depth + Slope.aspect.CTI.depth + Drained.elevation.slope.CTI.depth* + Elevation.slope.aspect.CTI.depth*	Block + Block.sample−location + Block.sample−location.depth	480	–	–
Estimated variance components:					
Random term	Component	Standard error	–	–	–
Block	0.00510	0.00560	–	–	–
Block.sample−location	−0.00267	0.01853	–	–	–
Residual variance model:					
Term	Factor	Model (order)	Parameter	Estimate	Standard error
Block. sample−location. depth	–	–	Residual variance	0.0602	0.01933
	Block	Identity	–	–	–
	Sample−location	Identity	–	–	–
	Depth	AR-1	Phi-1	0.6485	0.1134
Deviance and degrees of freedom:					
−245.51	380	–	–	–	–
Table of effects for constant (estimate and standard error):					
2.858	0.056	–	–	–	–
Table of effects for drained (drained, undrained and standard error of differences):					
< 0.000001	0.05443	0.07646	–	–	–
Table of effects for elevation (estimate and standard error):					
0.001154	0.043070	–	–	–	–
Table of effects for slope (estimate and standard error):					
−0.07467	0.05112	–	–	–	–
Table of effects for aspect (estimate and standard error):					
−0.000367	0.001936	–	–	–	–
Table of effects for CTI (estimate and standard error):					
−0.003508	0.044404	–	–	–	–
Table of effects for depth (0−2.5, 2.5−7.5, 7.5−15, 15−30 cm and average standard error of differences):					
< 0.0001	−0.4758	−1.0532	−1.7646	0.0437	–
Table of effects for drained.elevation (drained, undrained and standard error of differences):					
< 0.00001	0.05509	0.04997	–	–	–
Table of effects for drained.depth (0−2.5, 2.5−7.5, 7.5−15, 15−30 cm and average standard error of differences):					
Drained	< 0.00001	< 0.00001	< 0.00001	< 0.00001	–
Undrained	< 0.00001	0.02066	−0.01786	−0.03312	0.05964
Table of effects for elevation.depth (0−2.5, 2.5−7.5, 7.5−15, 15−30 cm and average standard error of differences):					
< 0.000001	0.00195	0.00936	0.03467	0.03723	–
Table of effects for drained.elevation.depth (0−2.5, 2.5−7.5, 7.5−15, 15−30 cm and average standard error of differences):					
Drained	< 0.00001	< 0.00001	< 0.00001	< 0.00001	–
Undrained	< 0.00001	0.01425	−0.00018	−0.01425	0.04336

*Significant fixed effect ($P < 0.05$).

AR-1, auto-regressive order 1 model.

All covariates centred. Not reported: estimated covariance models and tests for fixed effects. Partially reported: tables of effects. Covariance structures for random model, same as for total nitrogen (Table 2). CTI, compound topographic index.

Table 4 Linear mixed model for total carbon/total nitrogen (TC/TN) (response variate)

REML variance components analysis (final model):					
Response variate	Fixed model	Random model	Number of units	–	–
TC/TN	Constant + Elevation* + CTI* + Depth* + Elevation.depth*	Block + Block.sample–location + Block. sample–location.depth	480	–	–
Estimated variance components:					
Random term	Component	Standard error	–	–	–
Block	0.004	0.020	–	–	–
Block.sample–location	−0.649	0.395	–	–	–
Residual variance model:					
Term	Factor	Model (order)	Parameter	Estimate	Standard Error
Block.sample–location.depth	–	–	Residual variance	1.814	0.437
	Block	Identity	–	–	–
	Sample–location	Identity	–	–	–
	Depth	AR-1	Phi-1	0.6248	0.0888
Deviance and degrees of freedom:					
516.33	467	–	–	–	–
Tests for fixed effects: sequentially adding terms to fixed model:					
Fixed term	Wald statistic	Numerator degrees of freedom	*F*-statistic	Denominator degrees of freedom	*P*
Elevation	5.64	1	5.64	7.5	0.047
CTI	4.74	1	4.74	113.0	0.032
Depth	414.77	3	137.92	272.5	<0.001
Elevation.depth	19.24	3	6.40	272.5	<0.001
Tests for fixed effects: dropping individual terms from full fixed model:					
Fixed term	Wald statistic	Numerator degrees of freedom	*F*-statistic	Denominator degrees of freedom	*P*
CTI	4.74	1	4.74	113.0	0.032
Elevation.depth	19.24	3	6.40	272.5	<0.001
Table of effects for constant (estimate and standard error):					
10.17	0.10	–	–	–	–
Table of effects for elevation (estimate and standard error):					
0.01116	0.05886	–	–	–	–
Table of effects for CTI (estimate and standard error):					
0.1206	0.0554	–	–	–	–
Table of effects for depth (0–2.5, 2.5–7.5, 7.5–15, 15–30 cm and average standard error of differences):					
<0.001	−0.655	−1.166	−2.857	0.124	–
Table of effects for elevation.depth (0–2.5, 2.5–7.5, 7.5–15, 15–30 cm and average standard error of differences):					
<0.00001	−0.02864	0.01508	0.26682	0.07126	–

*Significant fixed effect ($P < 0.05$).

AR-1, auto-regressive order 1 model.

All covariates centred. Not reported: estimated covariance models. Covariance structures for random model, same as for total nitrogen (TN) (Table 2). CTI, compound topographic index.

close to 0‰, whereas less moisture in the soil of the drained plot decreases denitrification activity and the soil is less depleted in ^{15}N (Bedard-Haughn *et al.*, 2003). The δ^{15}N values and isotopic fractionation depend on nutrient supply and soil moisture, and δ^{15}N sample locations were not distributed evenly across each plot or block; therefore, they could have coincidentally followed flow patterns. Consequently, the complex distribution of δ^{15}N in soil samples at Rowden Moor might not have been represented adequately because of the sample design chosen. To determine whether this is so, different N pools, such as ammonium, nitrate and organic N, should be analysed in future.

The increase in δ^{13}C values with soil depth is common in cropped and grassland soils with C_3 vegetation (Wiesenberg *et al.*, 2004); it is related to the enhanced age and advanced degree of degradation of organic carbon in the soil with depth and improved contribution of root-derived organic matter at lower depths (Gocke *et al.*, 2011). The increase in δ^{15}N values with soil depth has been widely reported (Kerley & Jarvis, 1997). The depth gradient results from an increase in ^{15}N in SOM with depth, enhanced denitrification and NO_3^- leaching, an increase in stable carbon associated with clay (Kerley & Jarvis, 1997) and an increase in recalcitrant N (Högberg, 1997).

Table 5 Linear mixed model for δ^{15}N (response variate)

REML variance components analysis (final model):					
Response variate	Fixed model	Random model	Number of units	–	–
δ^{15}N	Constant + Elevation + Slope + Aspect + CTI + Depth* + Elevation.slope + Elevation.aspect + Elevation.CTI + Slope.CTI* + Aspect.CTI + Elevation.depth* + Slope.depth + CTI.depth + Elevation.slope.CTI* + Elevation.aspect.CTI* + Slope.CTI.depth*	Block + Block.sample–location + Block.sample–location.depth	480	–	–
Estimated variance components:					
Random term	Component	Standard error	–	–	–
Block	0.0307	0.0317	–	–	–
Block.sample–location	−0.2002	0.2263	–	–	–
Residual variance model:					
Term	Factor	Model (order)	Parameter	Estimate	Standard error
Block.sample–location.depth	–	–	Residual variance	0.746	0.241
	Block	Identity	–	–	–
	Sample–location	Identity	–	–	–
	Depth	AR-1	Phi-1	0.7087	0.0946
Deviance and degrees of freedom:					
192.77	449	–	–	–	–
Table of effects for constant (estimate and standard error):					
3.079	0.096	–	–	–	–
Table of effects for elevation (estimate and standard error):					
0.1594	0.0549	–	–	–	–
Table of effects for slope (estimate and standard error):					
−0.1197	0.0789	–	–	–	–
Table of effects for aspect (estimate and standard error):					
−0.00380	0.07887	–	–	–	–
Table of effects for CTI (estimate and standard error):					
−0.07905	0.07318	–	–	–	–
Table of effects for depth (0–2.5, 2.5–7.5, 7.5–15, 15–30 cm and average standard error of differences):					
<0.001	0.587	1.740	3.031	0.072	–
Table of effects for elevation.depth (0–2.5, 2.5–7.5, 7.5–15, 15–30 cm and average standard error of differences):					
<0.0001	−0.0733	−0.2219	−0.2218	0.0428	–

*Significant fixed effect ($P < 0.05$).

AR-1, auto-regressive order 1 model.

All covariates centred. Not reported: estimated covariance models and tests for fixed effects. Partially reported: tables of effects. Covariance structures for random model, same as for total nitrogen (TN) (Table 2). CTI, compound topographic index.

The value of topographic and spatial information

The LMM results indicated complex interactions amongst the topographic covariates in relation to variation in plant community and soil chemistry; therefore, future work should explore these processes in more detail. It may be worthwhile to reformulate slope and aspect because the circular nature of the latter does not suit its direct application in a statistical model (Mardia & Jupp, 2000). Topographic exposure (TOPEX) scores derived from the DEM could also be considered because these data can distinguish high from low ground in terms of exposure to wind, evapotranspiration and cooling of the soil surface (Pyatt, 1969).

All LMMs were specified with spatially correlated random effects; however, only those for species diversity retained a spatial element, suggesting that topographic data could act as covariates. It might have been useful to incorporate similar covariates related to soil type, soil texture, water holding capacity and geological substrate. A further possible limitation of the current study was the choice of soil depths because potentially different soil horizons are included in the individual depth increments. Future studies should consider these comments at the sample design stage.

Conclusions

For the plant community: hypothesis (i), that species diversity depends on topography because moisture steadily increases downslope, was supported and hypothesis (ii), that drained plots decrease species diversity, was refuted. For soil chemistry: hypothesis (i),

Table 6 Linear mixed model for δ^{13}C (response variate). All covariates centered

REML variance components analysis (final model):					
Response variate	Fixed model	Random model	Number of units	–	–
δ^{13}C	Constant + Drained* + Elevation* + Slope + Aspect + CTI + Depth* + Drained.elevation + Elevation.slope + Drained.aspect* + Elevation.aspect + Slope.aspect + Drained.CTI + Elevation.CTI + Slope.CTI + Drained.depth + Elevation.depth* + Slope.depth + Aspect.depth + CTI.depth + Drained.elevation.aspect* + Elevation.slope.CTI + Elevation.slope.depth + Slope.aspect.depth* + Drained.CTI.depth* + Elevation.CTI.depth + Slope.CTI.depth + Elevation.slope.CTI.depth*	Block + Block.sample–location + Block.sample–location.depth	480	–	–
Estimated variance components:					
Random term	Component	Standard error	–	–	–
Block	0.00158	0.00258	–	–	–
Block.sample–location	0.01260	0.00299	–	–	–
Residual variance model:					
Term	Factor	Model (order)	Parameter	Estimate	Standard error
Block.sample–location.depth	–	–	Residual variance	0.0135	0.00193
	Block	Identity	–	–	–
	Sample–location	Identity	–	–	–
	Depth	AR-1	Phi-1	0.3445	0.0956
Deviance and degrees of freedom:					
−1014.02	424	–	–	–	–
Table of effects for constant (estimate and standard error):					
1.381	0.032	–	–	–	–
Table of effects for drained (drained, undrained and standard error of differences):					
< 0.000001	0.09339	0.04403	–	–	–
Table of effects for elevation (estimate and standard error):					
0.02498	0.02183	–	–	–	–
Table of effects for slope (estimate and standard error):					
−0.01367	0.01941	–	–	–	–
Table of effects for aspect (estimate and standard error):					
0.00071	0.00141	–	–	–	–
Table of effects for CTI (estimate and standard error):					
−0.03252	0.02488	–	–	–	–
Table of effects for depth (0–2.5, 2.5–7.5, 7.5–15, 15–30 cm and average standard error of differences):					
< 0.0001	**0.0773**	0.2822	0.4423	0.0210	–
Table of effects for drained.elevation (drained, undrained and standard error of differences):					
< 0.00001	−0.03687	0.02652	–	–	–
Table of effects for drained.depth (0–2.5, 2.5–7.5, 7.5–15, 15–30 cm and average standard error of differences):					
Drained	< 0.00001	< 0.00001	< 0.00001	< 0.00001	–
Undrained	< 0.00001	−0.01904	0.00799	0.01171	0.02962
Table of effects for elevation. depth (0–2.5, 2.5–7.5, 7.5–15, 15–30 cm and average standard error of differences):					
< 0.00001	0.00050	0.02275	0.05367	0.00868	–

*Significant fixed effect ($P < 0.05$).

AR-1, auto-regressive order 1 model.

Not reported: estimated covariance models and tests for fixed effects. Partially reported: tables of effects. Covariance structures for random model, same as for total nitrogen (TN) (Table 2). CTI, compound topographic index.

that TN, TC, TC/TN, $\delta^{15}N$ and $\delta^{13}C$ depend on soil depth, with TN, TC and TC/TN decreasing with depth, whereas $\delta^{15}N$ and $\delta^{13}C$ increase, was supported; hypothesis (ii), that TN, TC, TC/TN, $\delta^{15}N$ and $\delta^{13}C$ depend on topography, with all properties decreasing downslope, was supported; hypothesis (iii), that enhanced mineralization of TN and TC contents decrease with drainage, was supported; hypothesis (iv), that TC/TN changes with drainage, was refuted and hypothesis (v), that $\delta^{15}N$ and $\delta^{13}C$ values increase with drier soil conditions (i.e. in drained plots), was refuted. For all soil chemistry properties, direct spatial effects were considered, but in each case, they could be adequately represented by topographic effects. Meanwhile, plant community (through species diversity) was the only variable to require a spatial term in its statistical modelling. A shift in the plant community from a *Lolium perenne*-dominated grassland with numerous patches of *Juncus* species towards one with *Lolium perenne* and *Trifolium repens* was also observed.

A direct effect of drainage on observed differences in soil TN, TC contents and stable isotope composition between drained and undrained plots was not conclusive because of limitations of the sample design. Nevertheless, the results from this study are of value because they can be used to guide the sample design of any future study on the Rowden Moor site. This study does confirm, however, the close coupling of C and N in soil profiles and the high-resolution data and statistical analysis tentatively point to their dependence on plant biodiversity and other factors such as drainage.

Although, this study relies on the specific artificial drainage set-up at Rowden Moor, the general trends are striking and should enable the transferability of outcomes to similar long-term drainage settings. However, overall agricultural soil management plays a major role and experimental studies for other climatic and topographic conditions are required to determine the full transferability of our results to other soil types and conditions.

Acknowledgements

We are grateful to the German Academic Exchange Service (DAAD) and the British Council for funding the personnel exchange programme ARC within the project 'Influence of water on preservation of lipids in soil'. As part of this project, Martina Gocke, Christian Neugebauer, Christine Hellerström and Matthieu Bourdenet contributed to sampling and sample preparation. Research was also supported by the Biotechnology and Biological Sciences Research Council (BBSRC BB/J004308/1) for the North Wyke Farm Platform.

References

Abid, M. & Lal, R. 2008. Tillage and drainage impact on soil quality: I. Aggregate stability, carbon and nitrogen pools. *Soil & Tillage Research*, **100**, 89–98.

Balesdent, J., Mariotti, A. & Guillet, B. 1987. Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. *Soil Biology & Biochemistry*, **19**, 25–30.

Bedard-Haughn, A., van Groenigen, J.W. & van Kessel, C. 2003. Tracing ^{15}N through landscapes: potential uses and precautions. *Journal of Hydrology*, **272**, 175–190.

Bennie, J., Hill, M.O., Baxter, R. & Huntley, B. 2006. Influence of slope and aspect on long-term vegetation change in British chalk grasslands. *Journal of Ecology*, **94**, 355–368.

Carlsson, G. & Huss-Danell, K. 2003. Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil*, **253**, 353–372.

Chen, H., Hou, R., Gong, Y., Hongwen, L., Fan, M. & Kuzyakov, Y. 2009. Effects of 11 years of conservation tillage on soil organic matter fractions in wheat monoculture in Loess Plateau of China. *Soil & Tillage Research*, **106**, 85–94.

Chou, W.W., Silver, W.L., Jackson, R.D., Thompson, A.W. & Allen-Diaz, B. 2008. The sensitivity of annual grassland carbon cycling to the quantity and timing of rainfall. *Global Change Biology*, **14**, 1382–1394.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Plamboeck, A.H., Templer, P.H. & Tu, K.P. 2002. Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics*, **33**, 507–559.

Dixon, E.R., Blackwell, M.S.A., Dhanoa, M.S., Berryman, Z., de la Fuente Martinez, N., Junquera, D. *et al.* 2010. Measurement at the field scale of soil $\delta^{13}C$ and $\delta^{15}N$ under improved grassland. *Rapid Communications in Mass Spectrometry*, **24**, 511–518.

Don, A., Scholten, T. & Schulze, E.-D. 2009. Conversion of cropland into grassland: implications for soil organic-carbon stocks in two soils with different texture. *Journal of Plant Nutrition and Soil Science*, **172**, 53–62.

Dungait, J.A.J., Hopkins, D.W., Gregory, A.S. & Whitmore, A.P. 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. *Global Change Biology*, **18**, 1781–1796.

Elgersma, A. & Hassink, J. 1997. Effects of white clover (Trifolium repens L.) on plant and soil nitrogen and soil organic matter in mixtures with perennial ryegrass (Lolium perenne L.) *Plant and Soil*, **197**, 177–186.

Frank, D.A., Depriest, T., McLauchlan, K. & Risch, A.C. 2011. Topographic and ungulate regulation of soil C turnover in a temperate grassland ecosystem. *Global Change Biology*, **17**, 495–504.

Fry, B. 2006. *Stable Isotope Ecology*. Springer, New York.

Gessler, P.E., Moore, I.D., McKenzie, N.J. & Ryan, P.J. 1995. Soil landscape modelling and spatial prediction of soil attributes. *International Journal of Geographical Information Systems*, **9**, 421–432.

Gocke, M.I., Pustovoytov, K., Kühn, P., Wiesenberg, G.L.B., Löscher, M. & Kuzyako, Y. 2011. Carbonate rhizoliths in loess and their implications for paleoenvironmental reconstruction revealed by isotopic composition: $\delta^{13}C$, ^{14}C. *Chemical Geology*, **283**, 251–260.

Gregory, A.S., Dungait, J.A.J., Watts, C.W., Bol, R., Dixon, E.R. & Whitmore, A.P. 2016. Long-term management changes topsoil and subsoil organic carbon and nitrogen dynamics in a temperate agricultural system. *European Journal of Soil Science*, **67**, 421–430.

Grime, J.P., Fridley, J.D., Askew, A.P., Thompson, K., Hodgson, J.G. & Bennett, C.R. 2008. Long-term resistance to simulated climate change in an infertile grassland. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 10028–10032.

Grootjans, A.P., Hunneman, H., Verkiel, H. & Van Andel, J. 2005. Long-term effects of drainage on species richness of a fen meadow at different spatial scales. *Basic and Applied Ecology*, **6**, 185–193.

Handley, L.L., Austin, A.T., Stewart, G.R., Robinson, D., Scrimgeour, C.M., Raven, J.A. *et al.* 1999. The ^{15}N natural abundance ($\delta^{15}N$) of ecosystem sample reflects measures of water availability. *Australian Journal of Plant Physiology*, **26**, 185–199.

Harrod, T.R. & Hogan, D.V. 2008. *The Soils of North Wyke and Rowden*. Unpublished report to North Wyke Research, revised edition of original report by T.R. Harrod, Soil Survey of England and Wales (1981). North Wyke Research, Okehampton, Devon.

Herzon, I. & Helenius, J. 2008. Agricultural drainage ditches, their biological importance and functioning. *Biological Conservation*, **141**, 1171–1183.

Hill, M.O. 1973. Diversity and evenness: a unifying notation and its consequences. *Ecology*, **54**, 427–432.

Högberg, P. 1997. Tansley Review No 95. ^{15}N natural abundance in soil–plant systems. *New Phytologist*, **137**, 179–203.

IPCC 2007. *Climate Change 2007: The Physical Science Basis. Working Group 1 Report*. Cambridge University Press, Cambridge.

IUSS Working Group WRB. 2006. *World Reference Base for Soil Resources 2006*. Second Edition. World Soil Resources Reports No 103, FAO, Rome, Italy.

Jentsch, A., Kreyling, J., Elmer, M., Gellesh, E., Glaser, B., Grant, K. *et al.* 2011. Climate extremes initiate ecosystem-regulating functions while maintaining productivity. *Journal of Ecology*, **99**, 689–702.

Jobbágy, E.G. & Jackson, R.B. 2001. The distribution of soil nutrients with depth: global patterns and the imprint of plants. *Biogeochemistry*, **53**, 51–77.

Kerley, S.J. & Jarvis, S.C. 1997. Variation in ^{15}N natural abundance of soil, humic fractions and plant materials in a disturbed and an undisturbed grassland. *Biology and Fertility of Soils*, **24**, 147–152.

Lal, R. 2004. Agricultural activities and the global carbon cycle. *Nutrient Cycling in Agroecosystems*, **70**, 103–116.

Mardia, K.V. & Jupp, P.E. 2000. Directional Statistics. *Wiley Series in Probability and Statistics*. John Wiley & Sons, Chichester.

Morris, J. 1989. Land drainage: agricultural benefits and environmental impacts. *Water and Environment Journal*, **3**, 551–557.

Murtagh, F. & Legendre, P. 2014. Ward's hierarchical agglomerative clustering method: which algorithms implement Ward's criterion? *Journal of Classification*, **31**, 274–295.

Piepho, H.P., Buchse, A. & Emrich, K. 2003. A hitchhikers guide to mixed models for randomized experiments. *Journal of Agronomy and Crop Science*, **189**, 310–322.

Pyatt, D.G. 1969. *Guide to the Site Types of North and Mid-Wales*. Forestry Commission, Forest Record No 69. Her eMajesty's Setationary Office, London.

Robinson, D., Handley, L.L., Scrimgeour, C.M., Gordon, D.C., Forster, B.P. & Ellis, R.P. 2000. Using stable isotope natural abundances (δ^{15}N and δ^{13}C) to integrate the stress responses of wild barley (*Hordeum spontaneum* C. Koch.) genotypes. *Journal of Experimental Botany*, **51**, 41–50.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A. *et al.* 2011. Persistence of soil organic matter as an ecosystem property. *Nature*, **478**, 49–56.

Soussana, J.-F., Loiseau, P., Vuichard, N., Ceschia, E., Balesdent, T., Chevallier, T. *et al.* 2004. Carbon cycling and sequestration opportunities in temperate grasslands. *Soil Use and Management*, **20**, 219–230.

VSN International 2015. *GenStat for Windows 18th Edition*. VSN International Ltd, Hemel Hempstead.

Wiesenberg, G.L.B., Schwarzbauer, J., Schmidt, M.W.I. & Schwark, L. 2004. Source and turnover of organic matter in agricultural soils derived from n-alkane/n-carboxylic acid compositions and C-isotope signatures. *Organic Geochemistry*, **35**, 1371–1393.