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RIPARIAN BUFFER STRIPS AS A MULTIFUNCTIONAL TOOL IN AGRICULTURAL LANDSCAPES

Relationships between Soil Physicochemical, Microbiological Properties, and Nutrient Release in Buffer Soils Compared to Field Soils

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The retention of nutrients in narrow, vegetated riparian buffer strips (VBS) is uncertain and underlying processes are poorly understood. Evidence suggests that buffer soils are poor at retaining dissolved nutrients, especially phosphorus (P), necessitating management actions if P retention is not to be compromised. We sampled 19 buffer strips and adjacent arable field soils. Differences in nutrient retention between buffer and field soils were determined using a combined assay for release of dissolved P, N, and C forms and particulate P. We then explored these differences in relation to changes in soil bulk density (BD), moisture, organic matter by loss on ignition (OM), and altered microbial diversity using molecular fingerprinting (terminal restriction fragment length polymorphism [TRFLP]). Buffer soils had significantly greater soil OM (89% of sites), moisture content (95%), and water-soluble nutrient concentrations for dissolved organic C (80%), dissolved organic N (80%), dissolved organic P (55%), and soluble reactive P (70%). Buffer soils had consistently smaller bulk densities than field soils. Soil fine particle release was generally greater for field than buffer soils. Significantly smaller soil bulk density in buffer soils than in adjacent fields indicated increased porosity and infiltration in buffers. Bacterial, archaeal, and fungal communities showed altered diversity between the buffer and field soils, with significant relationships with soil BD, moisture, OM, and increased solubility of buffer nutrients. Current soil conditions in VBS appear to be leading to potentially enhanced nutrient leaching via increasing solubility of C, N, and P. Manipulating soil microbial conditions (by management of soil moisture, vegetation type, and cover) may provide options for increasing the buffer storage for key nutrients such as P without increasing leaching to adjacent streams.

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Riparian vegetated buffer strips (VBS) comprise a border physically separating the agricultural activities within the field from the banks of the stream, or river. They are defined as a zone of no cultivation, grazing, or agrochemical application. Vegetated buffer strips have become a common option in the management of diffuse pollution and appear in ~70% of Europe-wide river basin management plans for the European Union (EU) Water Framework Directive (Eureau, personal communication, 2010). Buffer design varies spatially along river systems but also by country according to local policy, incentives, and agricultural landscapes. However, generally in EU countries, VBS are narrow, unmanaged borders, in which grow a succession of grasses, herbs, and woodier shrubs, then trees. In Scotland, the minimum statutory requirements for VBS state "no land shall be cultivated for a crop that is within 2 m of any surface water or wetland" (GBRA, 2008, p. 6).

Recent studies and reviews have shown varying efficiencies of VBS in retaining sediment and nutrients (Liu et al., 2008; Hoffmann et al., 2009; Collins et al., 2009). A review by Collins et al. (2009) noted retention efficiency values of 30 to 85% for 1- to 3-m widths and 80% for 6-m in the case of total P. Efficiencies were also drastically reduced for N species below widths of 10 m. The ability of buffers to trap sediments and particulate nutrients mobilized from field slopes depends on spatially heterogeneous pathways where flow becomes concentrated due to topography and development of erosion features. Despite this variability, VBS generally perform well in particle retention due to enhanced infiltration brought about by rooting from permanent plant cover (Cooper et al., 1995). However, the uncertainties in VBS function seem to be for dissolved nutrients. Indeed, Cooper et al. (1995, p. 65) concluded that "set-aside" "led to the development of a zone likely to supply runoff that is depleted in sediment-bound nutrients and dissolved N but enriched in dissolved P." Today, this uncertainty with respect to P functioning still exists. Collins et al. (2009) showed efficiencies for dissolved P of −83% (i.e., net losses) to +95%. Buffer P release has been shown in nutrient budget studies of field and buffer plots by Uusi-Kämppä

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Abbreviations: BD, dry bulk density; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorus; OM, organic matter; PC, principal component; PCR, polymerase chain reaction; RDA, redundancy analyses; SRP, soluble reactive phosphorus; TDP, total dissolved phosphorus; TRF, terminal restriction fragment; TRFLP, terminal restriction fragment length polymorphism; VBS, vegetated buffer strips.

(2005) and Dillaha et al. (1988). While denitrification leads to permanent removal of N from the terrestrial ecosystem as unreactive N_2, no long-term loss pathway exists for P from VBS. Soil P sorption can only provide a short-term sink and is prone to saturation, or altered conditions leading to desorption. Recent laboratory studies into geochemical sorption processes performed in the United States (Schroeder and Kovar, 2008) and the United Kingdom (Stutter et al., 2009a) have observed enhanced P desorption from VBS relative to soils in adjacent fields. Abiotic sorption processes may be regulated by P saturation of the soil sorption surfaces, or changes in the sorption state of the surfaces. The latter is well known to release P via reductive dissolution of Fe and Al sorbing phases as soils become anoxic (Litaor et al., 2005; Chacon et al., 2008). Trapped sediments may also become buried in VBS. Stutter et al. (2009a) showed this did not increase the P saturation of VBS soils after incorporation of sediments. Hence, without further biogeochemical processing, the incorporation of sediments in VBS soils may constitute a sink for P.

Microbiogical processes are implicated in P release by reductive dissolution of Fe(III) oxides under relatively severe changes in soil redox state (Hoffmann et al., 2009). However, the transition of VBS development from previous cropland may involve changes in soil–plant–microbial nutrient cycling, the implications of which are only now becoming researched with relation to nutrient retention. Studies have shown enhanced degradation rates for pesticides in buffer, relative to field soils (Staddon et al., 2001; Krutz et al., 2006). A recent comprehensive review of nutrient mobilization and transport in buffers by Vidon et al. (2010) concentrated on coupled geochemical and hydrological processes for P, while appraising the more extensive evidence of biogeochemical processes for N and C cycling. Phosphorus leaching from VBS may have previously been considered dominated by geochemical processes, but Uusi-Kämppä (2005) reported that net release of soluble P from VBS coincided with periods of vegetation senescence. There may also be P release from humic-Al(Fe)–P complexes and indirectly from the organic matter during decomposition processes (Giesler et al., 2005). Concern that buffer organic matter cycling might promote dissolved organic C (DOC) leaching to streams prompted a recent U.S. catchment study (Veum et al., 2009), but in this case that suggestion was not proven. Since the biogeochemical cycling of N and C have been coupled in riparian soils and streamwaters (Knowles, 2005; Bernhardt and Likens, 2002; Tank et al., 2000), it makes sense to extend this to include P.

Our recent study (Stutter et al., 2009a) implied that enhanced biological processing was implicated in solubilizing P for a limited number of VBS soils within a single catchment. It was proposed that enhanced P leaching corresponded with a shift in the overall microbial community. Here we aim to (i) compare VBS soils with adjacent cropped field soils at wider range of sites/soil parent materials; (ii) investigate interactions between changing soil physicochemical properties (organic matter, moisture content, bulk density, particle size, water-dispersed fine sediment release, and dissolved C, N, P, and particulate P release); and (iii) relate these properties to changes in bacterial, archeal, and fungal communities over short environmental gradients from field to stream bank. It may be inappropriate to presume that buffer soils will sequester a range of nutrient forms, input from upslope, simply because they are left uncultivated and unfertilized. Therefore, any widespread and consistent link between increased solubility of forms of C, N, and P and altered microbial status of buffer soils will benefit future mechanistic studies investigating how we may manipulate buffer biological conditions to increase nutrient storage and combat leaching losses.

Materials and Methods

Sites and Sampling

Soils were sampled during spring 2010 from 19 sites across three arable agricultural regions of Scotland (see Supplemental Fig. S1). Sites covered three soil parent material types/groups within the following five soil associations (with number of sites in each group) and dominant characteristics:

1. Balrownie/Corby/Mountboy Associations (n = 4), Old Red Sandstone till to fluvial glacial sands and gravels, sandy loam;
2. Tarves Association (n = 10), mixed acid–basic, metamorphic–igneous till, sand–clay loam;
3. Rowanhill Association (n = 5), sandstone, shales, limestone, sandy silt loam.

Together these parent materials cover 25% of Scotland's cropland (Supplemental Fig. S1). Sites were selected on the basis of the presence of a VBS (minimum width 2 m) between arable land and a first- or second-order stream.

Before sampling, sites were surveyed for VBS width, land use, slope, fencing or in-field erosion control measures (e.g., contour plowing) presence, and extent of field erosion according to Kirkbride and Reeves (1993) using three categories (low—no visible signs; medium—localized sheetwash and colluvial deposition; severe—rill development and resedimentation across significant areas). Details of the site properties are given in Supplemental Table S1. Land use in the fields adjacent to the sampled buffers was dominantly winter cereals (n = 14), with spring cereals (n = 4) and brassicas (n = 1). Surveys suggested site erosion extents were limited, with categorization as: no visible signs (n = 8), moderate (n = 10), and severe (n = 1; including eroded material reaching the stream). Buffer widths were 1 to 12 m, median 3 m. Three sites had bare soil (recently tilled surfaces), with the majority under crop or stubble at the time. We have no information on fertilizer input history, but it is expected that these are high-P-status arable soils, and Scottish agronomic guidance states such soils receive only maintenance inputs of inorganic P fertilizer (i.e., balancing crop offtake rates). We cannot date the establishment of the VBS, but their mature grass to shrub vegetation would suggest periods of >3 yr.

Soils were sampled in triplicate, spaced 5 m apart parallel to the VBS, (i) on the cropped field side (10 m upslope of the buffer interface) and (ii) within the buffer (the minimum of either 2 m from the VBS upslope edge, or half the width), hereby referred to as *field* and *buffer* samples, respectively. Soils were sampled by gently inserting a steel ring core (6-cm diameter, 6-cm depth) into the soil (upper edge of core at soil profile surface), cutting through roots, if necessary, to avoid compression. Soils in VBS had much greater surface rooting than field soils. Individual cores were bagged, transported to the laboratory that day, and maintained at field moisture, in the dark at <4°C before preparation (within 5 d).

Physicochemical Analyses

Soils were removed from cores, roots were picked out manually, then soils were sieved field-moist to <2 mm, and soil and stone contents weighed separately. A 20-g subsample removed for chemical and microbial characterization was maintained field-moist. The remaining soil was dried at 105°C, and soil moisture and bulk density (scaled to account for the subsampling) was determined gravimetrically. Particle size distributions of soils (<2-mm air-dried fractions) were determined following dispersion (16 h end-over-end shaking in 1.6 mol L^{-1} ammonium solution) by laser diffraction (Mastersizer 2000 with HydroG dispersion unit, Malvern Instruments, Malvern, UK). Loss on ignition was determined gravimetrically on 1-g samples of each soil (triplicate analytical replicates) following ignition at 550°C for 1 h. These results are hereby termed *organic matter* (OM) content.

Field-moist soils underwent a combined assay of dissolved and particulate nutrient release and aggregate stability (see Fig. 1). Changes in these properties associated with drying and rewetting were negated by using field-moist samples. Ten-gram subsamples of moist soils were combined with a sufficient volume of 1 mM NaCl to give a 1:10 oven-dried mass (g):solution (mL) ratio and equilibrated for 16 h at 20°C (in darkness) on an orbital shaker. Equilibrations were then shaken vigorously by hand and left to settle at 20°C for exactly 31 min. At this time two 5-mL aliquots were carefully drawn off using a fine glass pipette from 5 cm under the liquid surface. Under these temperature, depth, and time conditions this sampled the <6-μm particles (i.e., fine silt plus clay by Atterberg particle size classes). This was calculated according to Stoke's law (Gee and Bauder, 1986) assuming a soil density of 2650 kg m^{-3}. One of these aliquots was placed onto the center of a GF/F filter paper and sediment mass determined gravimetrically following drying at 105°C. The second 5-mL aliquots (unfiltered suspensions) underwent a manual persulfate digestion (Williams et al., 1995) at 110°C for 30 min, and the total P load was determined as soluble molybdate reactive P (SRP) by automated colorimetry (San^{++}, Skalar, Breda, the Netherlands). The remaining original suspensions were then filtered to <0.45 μm and filtrates analyzed for SRP, NO_3–N, and NH_4–N, and following an automated digestion procedure, total dissolved N (TDN), total dissolved P (TDP), and DOC analyses by colorimetry (San^{++}, Skalar). Dissolved organic N (DON) and dissolved organic P (DOP) were determined by difference as DON = TDN − (NO_3–N + NH_4–N) and DOP = TDP − SRP. Detection limits were 1 μg L^{-1} for SRP and TDP, and 1, 0.1, and 0.5 mg L^{-1} for NO_3–N, NH_4–N, and DOC.

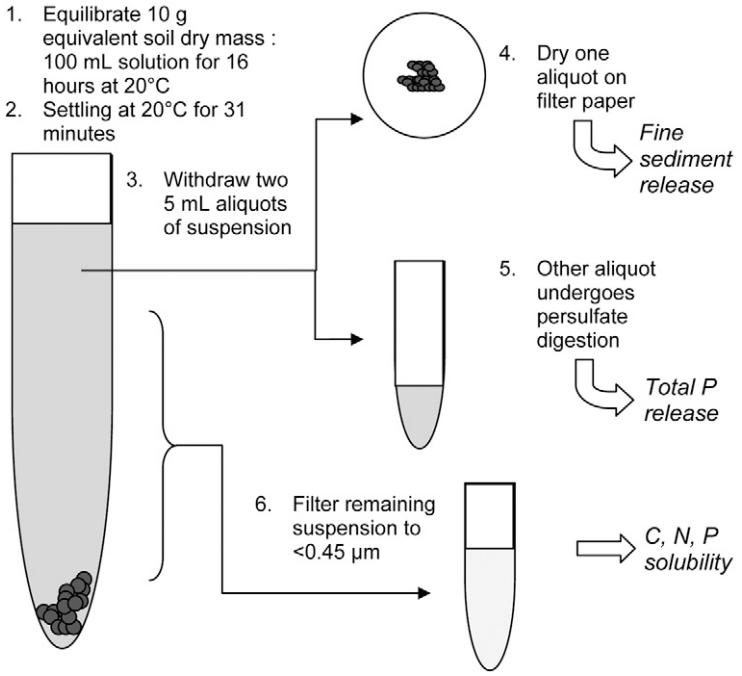

Fig. 1. Schematic of combined testing of nutrient release, aggregate stability, and total P mobilization from field-moist soil samples.

Microbiological Community Fingerprinting by TRFLP Analysis

Terminal restriction fragment length polymorphism (TRFLP) analysis is a robust, high-throughput method for fingerprinting and studying the distribution, structure, and diversity of microbial communities (Liu et al., 1997). A TRFLP analysis has been applied to the study of fungal ribosomal genes and bacterial and archaeal 16S rRNA genes (Schütte et al., 2008). DNA was extracted from 0.25 g of fresh soil samples using the PowerSoil-htp 96 Well Soil DNA isolation kit (Mo Bio, Carlsbad, CA) according to manufacturer's protocols. Isolated DNA was amplified with polymerase chain reaction (PCR) using a multiplex of primers specific for bacteria, archaea, and fungi. Multiple primers are used in the same PCR reaction to study different taxa (Schütte et al., 2008; Singh et al., 2006). The primers used are ITS1F (FAM; 5′-CTTGGTCATTTAGAGGAAGTAA-3′) fungal-specific ITS, 63F (VIC; 5′-AGGCCTAACACATGCAAGTC-3′), and 1087R (VIC; 5′-CTCGTTGCGGGACTTACCCC-3′) for bacterial and archaeal 16S rRNA genes. The PCR was performed on DyadDisciple Peltier Thermal Cycler (BioRad, Hercules, CA), using program consisting of 5 min at 95°C followed by 30 repeated cycles of (30 s at 94°C: denature, 30 s at 55°C: annealing, and 1 min at 72°C: elongation), then 10 min at 72°C and finally hold at 15°C. The PCR was stained with fluorescent dye ethidium bromide and visualized using 1% agarose gel that ran at 80 V for 80 min. The PCR product was cleaned up and purified to remove any unwanted reaction components using Mo Bio UltraClean-htp 96 Well PCR clean-up kit (Mo Bio, Carlsbad, CA) following the manufacturer's protocol. DNA was quantified using NanoDrop ND-1000 Spectrophotometer (NanoDrop, Wilmington, DE) before being digested with *Hha*1 restriction enzyme. A digestion mixture was prepared and incubated at 37°C for 3 h, then at 95°C for 10 min, and finally held at 10°C. Aliquots of 2 μL of each digested sample were used for fragment size analysis using Applied Biosystems 3130xl Genetic analyzer for TRFLP fingerprinting (Applied Biosystems, Carlsbad, CA). Analysis of TRFLP profiles were performed using GeneMapper software version 4.0 (Applied Biosystems, Carlsbad, CA). All TRFLP electropherograms were visually inspected and tabulated. A threshold of 30 to 550 bp was applied so true peaks can be determined from background noises.

Statistical Methods

Genstat v6 (VSN International, Oxford, UK) was used for statistical analyses. The sampling regime enabled testing differences between soil physical and chemical properties between field and buffer soils as a whole population (using ANOVA) and at individual sites (using individual site paired *t* tests and summing the numbers that were significant or not at $p < 0.05$). Two-way ANOVA was used (buffer vs. field and regions) with Tukey simultaneous testing (p level of 0.05). Physical and chemical properties were transformed, on the basis of Anderson–Darling testing $p > 0.05$), using Box–Cox transformations (to optimal lambda).

Structures of the three microbial communities were examined in two ways. First, data for all the terminal restriction fragments (TRFs) were analyzed by multivariate methods separately on the bacterial, archaeal, and fungal data sets. Principal components analyses were undertaken to reduce the dimensionality of the TRFs data to the first five principal components (PCs; PC1–PC5). To determine the effect of buffer vs. field soil differences and combining data from all sites (i) ANOVA was undertaken on PC1 and PC2, and (ii) overall MANOVA on PC1 to PC5. Correlation analyses (r) were undertaken between PC1, PC2, and environmental data. Second, TRFs of each of the bacterial, archaeal, and fungal data sets were ranked according to overall abundance across all samples. For the top 15 most abundant TRFs (Singh et al., 2009) (iii) ANOVA was performed to determine the strength of the effect of buffer vs. field soil, then (iv) correlation analyses (r) and visual plotting were used to evaluate responses of individual TRFs to environmental variables (soil and nutrient release properties). Since responses were dominantly linear, (v) redundancy analyses (RDA) were then performed on the 15 most abundant TRFs and environmental variables. Initially, all environmental factors were screened for significant correlations in the RDA with TRF scores, and biplots were limited to environmental variables that were significant at $p < 0.05$. The RDA ordination biplots show the weighted abundances of individual TRFs, the relative importance of environmental factors in explaining variation in TRF profiles (by length of arrow), and the degree of correlation between TRFs and environmental variables (by commonality in the angles from the plot origin). In processing and analyzing this TRFLP data we have followed standard protocols (Kennedy et al., 2005; Singh et al., 2006). The TRFLP analyses were undertaken on a subsample of soils from Sampling Regions 2 and 3 ($n = 90$), as these soils had been in storage too long to enable extraction of the DNA (>30 d).

Results

Site Properties

There were no relationships between any of the soil properties described below and buffer width, field slope, or erosion score (data not shown). A visual impression of the typical field and buffer setting can be gained from Fig. 2.

Soil Physicochemical Properties

Results in Fig. 3 show overall differences buffer vs. field and also testing for differences by sampling region/parent material by use of two-way ANOVA. Table 1 gives overall one-way ANOVA results and individual site *t*-test counts for buffer vs. field soils, pooling data across all regions. The ANOVA results (Table 1) revealed highly significant differences between buffer and field soil for properties of bulk density, percent clay content, moisture content, and OM contents. Several physical properties showed consistent differences between buffer vs. field soils at all sites according to individual site *t* tests. Dominantly (17, or 18 out of 19 sites), field soils had greater bulk densities (BD), smaller OM contents, and smaller moisture contents than buffers. In terms of particle size and fine sediment release under minimal dispersion the percent clay content (following dispersion) and water-dispersed fine silt + clay contents were generally greater in buffer than in field soils, although differences were not significant at many sites. Figure 3 shows the variation in these properties, accounting also for regional effects. Region/soil parent material was significant in the two-way ANOVA for BD, fine silt + clay content, and soil moisture ($p < 0.01$), but not OM content ($p = 0.24$). The BD was approximately 30% lower in buffer soils consistently across all regions. Buffer conditions increased soil moisture considerably (approximately double in Region 3 with the finer textured soils), and OM content nearly doubled in buffer compared to field soils (especially for Region 1).

Fig. 2. Typical narrow, unmanaged vegetated buffer strips as required by diffuse pollution regulations in northeast Scotland.

Soil nutrient properties were significantly different between buffer and field soils (Table 1). Extracted concentrations of dissolved organic nutrients (DON, DOP, DOC) and SRP were dominantly greater in buffer than field soils. Nowhere (except two sites for P) did field soils release significantly greater concentrations of these nutrients. Concentration ranges for NO_3 and SRP were large (2–20 mg N L^{-1} and 0.1–1.8 mg P L^{-1}, respectively). Ratios in concentrations of DON (combined-site means 1.2 mg N L^{-1} buffer and 0.7 mg N L^{-1} field) to NO_3 (means 5.2 mg N L^{-1} buffer and 4.5 mg N L^{-1} field) were 0.3 (maximum 0.7) for buffers and 0.3 (maximum 1.6) for field soils. For DOP (means 0.18 mg P L^{-1} buffer and 0.11 mg P L^{-1} field) relative to SRP (means 0.48 mg P L^{-1} buffer and 0.23 mg N L^{-1} field) concentration ratios were 0.5 (maximum 2.4) for buffers and 0.7 (maximum 5.8) for field soils. Therefore, for N and P, processes in buffer soils generally elevated inorganic over organic nutrient forms. However, DOC was considerably greater in buffer (combined sites mean 9.1 mg C L^{-1}) than in field soils (mean 5.9 mg C L^{-1}). Region/parent material was significant in two-way ANOVA for all release of all nutrients ($p < 0.01$), with the

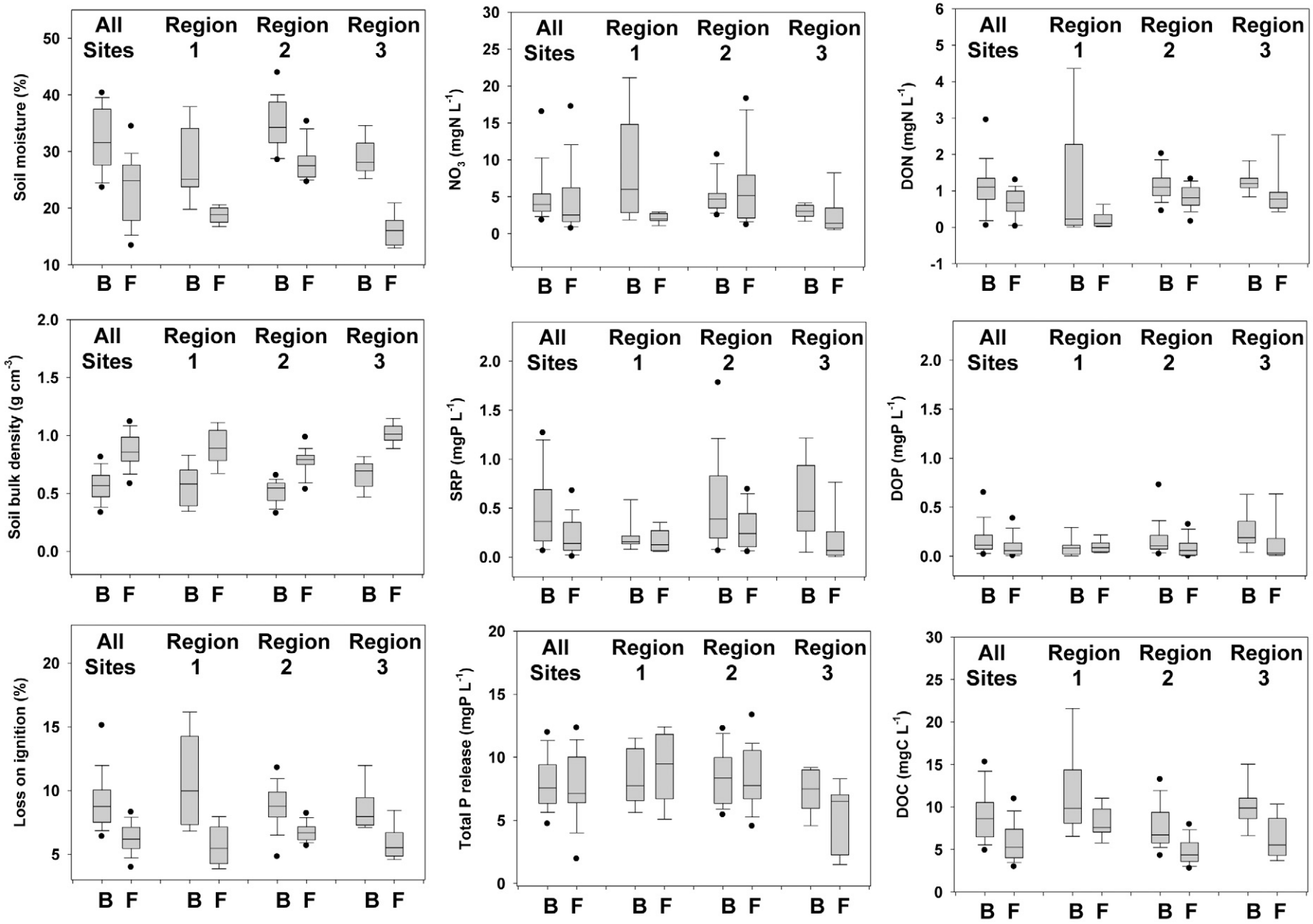

Fig. 3. Box (25, 50, and 75 percentiles) and whisker (5 and 95 percentiles) plots of the range of values in selected physicochemical properties comparing buffer (B) vs. cropped field (F) soil (all sites data and separately for the three sampled regions). DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorus; SRP, soluble reactive phosphorus.

Table 1. Summary statistics for soil physicochemical properties: (i) categories for individual site *t* tests (B, buffer $n = 3$; F, field $n = 3$) based on a significance threshold of $p < 0.05$, with (ii) overall significance given by ANOVA (buffer [$n = 57$] vs. field [$n = 57$]). Significant values are in italics. All units are concentrations as mg L^{-1}, except where stated.

Property†	Functional relevance	(i) Individual site *t* tests as number of sites in each result category			(ii) Overall significance by ANOVA
		Significant difference B > F	Significant difference F > B	No significant difference	B vs. F
BD (g cm^{-3})	Infiltration capacity	0	18	1	*0.000*
% sand	Indication of particle deposition/ erosion	5	4	10	0.352
% silt		5	5	9	0.135
% clay		0	8	11	*0.001*
Moisture content (%)	Influences biological processes	18	0	1	*0.000*
OM content (%)		17	0	2	*0.000*
DON‡	Organic nutrient release	11	0	8	*0.000*
DOP‡		10	2	7	*0.009*
DOC‡		15	0	4	*0.000*
NO_3‡	Inorganic nutrient release	8	5	6	*0.000*
SRP‡		14	2	3	*0.000*
Fine sediment release	Soil erosivity and P carrying capacity	3	8	8	*0.004*
Total P		7	5	7	0.422

† BD, dry bulk density; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorus; OM, organic matter; SRP, soluble reactive phosphorus.

‡ Data transformed by Box–Cox transformation before testing.

exception of DOP ($p = 0.11$). Comparisons between buffer and field soils were least consistent for NO_3. At eight out of 19 sites NO_3 concentrations were largest from buffers, but there was a strong interaction ($p < 0.01$) as Region 2 showed sites where field soils released greater NO_3 concentrations than adjacent buffers.

The assay to estimate fine sediment release (<6 μm, fine silt to clay particle sizes) showed a significant difference between buffer and field soils. Generally, when individual site *t* tests were performed, field soils released greater concentrations of fines than buffer soils, following the trend for greater percent clay particle size contributions. The total P release associated with this fine particle dispersion was not significantly different between the buffer and field soils and showed strong influence of region ($p < 0.001$). However, total P concentrations (3–14 mg P L^{-1} using the persulfate digestion extraction) were much larger than dissolved P release (Fig. 3).

Two relationships (Fig. 4) between solution and soil properties are highlighted to show differences between the buffer and field soil nutrient processes. In Fig. 4a, when buffer and field soils are considered together, a curved relationship is seen between concentrations of DON released in the leaching assay and soil moisture. But when separated, field soil showed a shallow gradient in DON release against soil moisture, compared to a much steeper gradient for the buffer sites (Fig. 4a). Second (Fig. 4b), there was an overall relationship for combined sites between DOC release against soil OM content ($R^2 = 0.26$; $p < 0.001$). This comprised a stronger relationship for separated buffer soils with greater concentrations of soil OM and DOC released.

Soil Microbiological Properties

The TRFLP analyses detected total numbers of unique DNA fragments of 117, 23, and 116 for bacteria, archaea, and fungi, respectively. The TRFs ranged in size (numbers of base pairs) from 41 to 544 for bacteria, 44 to 515 for archaea, and 36 to 483 for fungi.

Principal components analyses of all TRFs showed that the first five PCs combined explained 66, 95, and 54% of the sample variance for bacteria, archaea, and fungi, respectively. There was evidence of highly significant shifts in community diversity between buffer and field soils in the bacterial, archaeal, and fungal populations assessed using MANOVA on these first five PCs (all $p < 0.001$). Testing PC1 and PC2 showed bacterial diversity was overall significantly different between buffer and field (Table 2), but this effect was variable by site (included as a blocking factor). Bacterial PC1 correlated significantly with many soil properties: percent clay << NO_3 < moisture < BD < DON and most strongly with OM content and organic nutrient release. PC2 showed only a weak correlation with OM content. Archean diversity differed between buffer and field and community shifts correlated significantly with physical soil properties (particle size and BD). Fungal diversity differed between buffer and field soils and PC1 showed significant site effects. Fungal community shifts correlated significantly with moisture, BD, and OM content, especially for PC1, with a correlation of PC2 with SRP release.

Relationships were then explored between the 15 most abundant individual TRFs and soil property differences in the buffer vs. field data set using RDAs (summarized using biplots; Fig. 5). Abundance rankings for the top 15 TRFs in each of the bacterial, archaeal, and fungal data sets with individual TRFs *p* values for buffer vs. field are given in Supplemental Table S2. In all cases below, *p* values are described between soil properties and the dominant explanatory axis only (RDA1). For bacteria (Fig. 5a) NO_3 was strongly related to bacterial community structure ($p < 0.001$) with a positive impact on TRF174 and TRF175 and a negative impact on TRF72. Moisture ($p < 0.01$), OM content, and DOC (both $p < 0.001$) were related positively to TRF143 and negatively to TRF42. Conversely, BD ($p < 0.01$) was related negatively to TRF143 and positively to TRF42. The DON, DOP, and SRP showed a weak relationship ($p < 0.05$). The RDA for archaea (Fig. 5b) explained limited sample variance (6 and 2%), this being BD ($p < 0.001$) and clay ($p < 0.05$) negatively with TRF182. For fungi (Fig. 5c) many soil

properties were significant (with exceptions of NO_3, sand, and silt). Strongest relationships were OM content and moisture ($p < 0.001$) positively with TRF326 and negatively with TRF327 and TRF319. Conversely, BD ($p < 0.001$) related negatively to TRF326 and positively to TRF327 and TRF319. The DOP positively related to TRF328 ($p < 0.05$). These relationships between soil properties and individual TRFs agreed with overall relationships with PCs (Table 2).

Discussion

Buffer zones around streams are envisaged to provide a barrier to trap and retain nutrient losses from the surrounding agricultural land. Our results show enhanced DOC, DON, DOP, and SRP solubility from buffer soils relative to their adjacent cropland during short-term laboratory nutrient release assays using field-moist soils. This supports a growing body of evidence (Dillaha et al., 1988; Cooper et al., 1995; Uusi-Kämppä, 2005; Stutter et al., 2009a; see also this issue) that VBS show enhanced solubility of soil nutrients and hence greater potential for nutrient leaching directly adjacent to streams. This is converse to their perceived buffering effect. High concentrations of readily bioavailable inorganic and organic dissolved nutrient forms are of concern for water managers. The proximity of the VBS to the watercourse is one significant factor in the likelihood that these elevated in situ dissolved nutrient concentrations pose a risk of enhanced nutrient leaching. Another factor is increased hydraulic conductivities of VBS soils relative to cropland, which has previously been reported (Cooper et al., 1995; Seobi et al., 2005). Greater hydraulic conductivity would suggest that surface- and shallow soil–flow entering the buffer is readily transmitted through the buffer surface soil and likely to transfer any solubilized nutrients resulting from cycling processes to the stream. Our results confirmed consistently smaller BD for buffer compared with field soils. Small BD is often taken as an indicator of increased porosity, and in turn, of greater infiltration and hydraulic conductivity. However, there are often inverse relationships between soil BD and OM contents (Stutter et al., 2009b). Our study did not directly measure hydraulic conductivity, and this should be investigated more thoroughly in future. Greater infiltration capacity would suggest the trapping of fine eroded particles in the soil matrix (Hoffmann et al., 2009) and this is a desired primary function of buffers. However, we observed that both percent clay content by laser diffractometry (following chemical and ultrasonic dispersion) and the fine silt + clay–size particle release by the minimal-dispersion erodobility assay were generally greater for field than for buffer soils. This suggests either that fine material was stabilized by the OM in the buffers, or alternatively that the buffer is able to trap sand to coarser silt particles but that the sub–fine silt material is eroded through the buffer. If the latter were the case, it may be that the roughness and/or infiltration capacities are insufficient for these buffer width–slope combinations to deposit these fines. This would leave streams poorly protected from P and pathogens cotransported with soil fines but limit the input of P to the buffer soils from particulate forms.

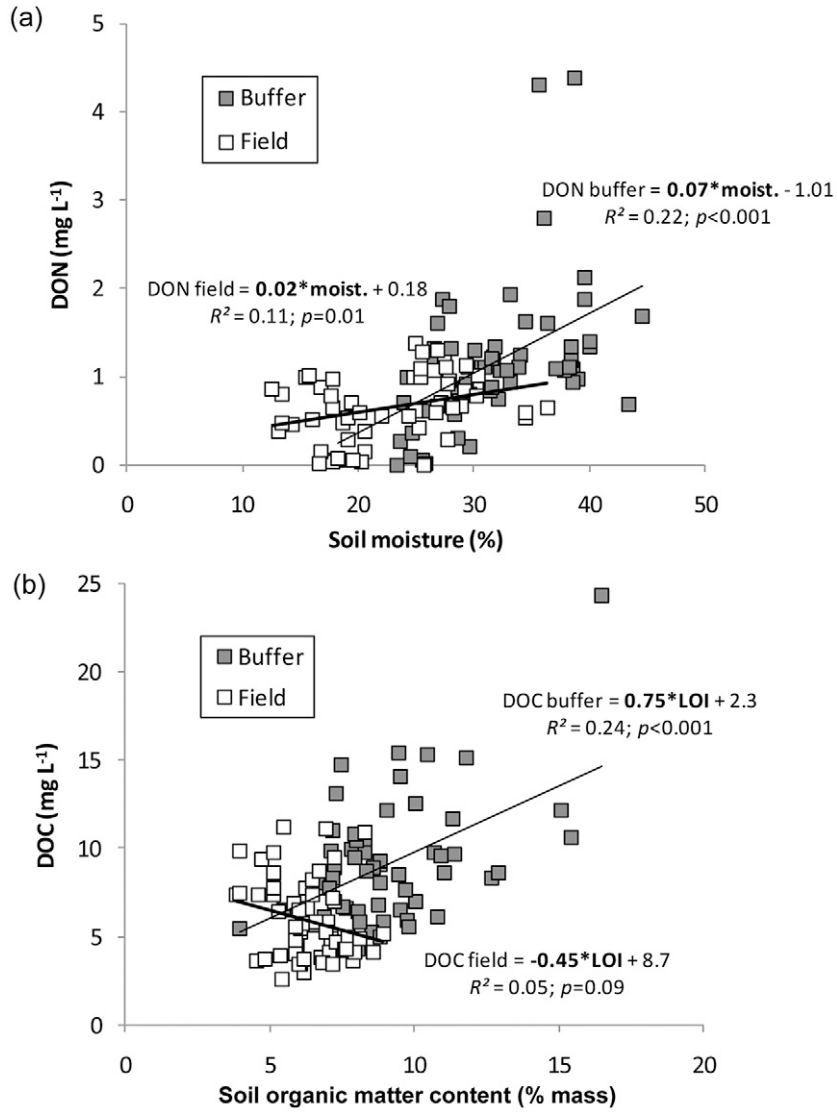

Fig. 4. Relationships for (a) dissolved organic nitrogen (DON) release with field soil moisture content and (b) dissolved organic carbon (DOC) release with soil loss on ignition plotted using different notation for buffer and field sites. Separate relationships are used to highlight differences between nutrient processing between buffer and field soil conditions.

The strongest enhancement of nutrient release in VBS relative to the cropped fields occurred for SRP and dissolved organic nutrients. The enhanced concentrations of organic nutrients in our release assay probably result from the mineralization of the accumulating soil OM in the buffer. We do not, however, know the age of the buffers and hence these rates of OM accumulation. The corresponding increases in mean SRP:DOP for the buffer soils indicated either mineralization of organic P, or that microbial processes, or sorption competition with organic acids have increased geochemical desorption (biotic interactions with abiotic processes). While an increase in buffer OM has positive implications for landscape C storage, the available C is the fuel for microbial cycling of nutrients, which may hasten their

Table 2. Significance (*p* values) of ANOVA into the effects of treatment (buffer vs. field) and site (blocking factor) and relationships with soil physicochemical properties (correlation coefficients). Significant values are in italics. All units are concentrations as mg L^{-1}, except where stated.

Property†	Bacteria		Archaea		Fungi	
	PC1‡	PC2	PC1	PC2	PC1	PC2
% variation explained	31.2	11.5	58.1	19.3	21.6	13.3
Buffer (B) vs. field (F)	*0.000*	*0.012*	*0.017*	*0.007*	*0.000*	*0.014*
Site	*0.000*	*0.000*	0.544	*0.001*	*0.000*	0.731
Interaction B vs. F × site	*0.002*	*0.001*	0.123	0.509	*0.007*	0.857
	PC1§	PC2§	PC1	PC2§	PC1§	PC2§
% clay	*0.037*	0.711	*0.013*	0.263	0.083	0.088
% silt	0.193	0.963	0.814	*0.034*	0.694	0.713
% sand	0.111	0.996	0.925	0.051	0.937	0.522
NO_3–N§	*0.005*	0.182	0.654	0.073	0.162	0.121
Organic N§	*0.001*	0.787	0.760	0.104	0.706	0.062
SRP§	0.055	0.893	0.089	0.087	0.691	*0.047*
Organic P§	*0.000*	0.277	0.336	0.647	0.294	0.155
DOC§	*0.000*	0.959	0.260	0.275	0.239	0.245
% moisture§	*0.004*	0.384	0.515	0.207	*0.000*	*0.042*
BD (g cm^{-3})§	*0.002*	0.380	*0.009*	*0.017*	*0.000*	*0.043*
LOI (%)	*0.000*	*0.047*	0.177	0.163	*0.000*	0.082
SPM	0.781	0.874	0.165	0.854	0.108	0.439

† BD, dry bulk density; DOC, dissolved organic carbon; LOI, loss on ignition; OM, organic matter; SRP, soluble reactive phosphorus; SPM, suspended particulate matter.

‡ PC, principal component.

§ Data transformed by Box–Cox transformation before testing.

leaching. Staddon et al. (2001, p. 1136) commented that "when VBS are established without tillage, a litter layer from decaying above-ground vegetation and massive network of roots alters patterns of OM accumulations that should substantially stimulate microbial processes." Ehlers et al. (2010) recently observed in in situ soil C, N, P spiking experiments that microbial growth rates depended on available P, but that available C constrained the potential size of the final microbial populations. Hence, microbial soil N and P cycling potential in buffers will be enhanced by the availability of this increased OM, where it comprises metabolically favorable forms. Additionally, Kögel-Knabner et al. (2008) showed that the presence of orthophosphate ligands increased desorption and bioavailability of available C forms in soils. Figure 4 suggests an enhanced rate of OM decomposition in the buffer soil and, together with greater overall OM contents, this likely explains the enhanced DOC, DON, and DOP concentrations (Kalbitz et al., 2000). The accessibility of the OM will be improved by the combined actions of bacterial–fungal decomposers. Hence, TRFLP methodologies are attractive since they explore diversity in the different components of the soil microfauna. Although we do not present process-investigations to firmly connect these microbial components to nutrient processing, the empirical relationships suggest that a range of microbes is implicated in explaining the greater nutrient release from buffer than field soils. Because our study aimed to connect biogeochemical and physical processes, we were careful to maintain field-moist soils for our leaching and erosion risk assay to counter arguments that the nutrient release was due to a drying–rewetting pulse variably between the buffer and field soils of initial different natural moisture conditions.

The overall principal components summary of the TRFLP data showed very significant shifts in microbial community diversity over the transition of field to buffer soil. It is a limitation of our study, however, that these data were not combined with microbial biomass and biomass C, N, and P pools. Future studies should include such results to improve process-understanding. Our methods show changes in microbial diversity, not biomass, but the latter have been reported before for VBS (Staddon et al., 2001). Analyses by TRFLP are rarely used in the literature to assess community shifts between different environments, across wide ecological gradients since it may be expected that drastic variation in conditions (wet to dry soils, very different soil types) greatly influence microbial communities. However, our data are interesting in that these community shifts occurred across short distances (<10 m) between a cropped field and adjacent VBS. We assume that the newly formed VBS soil previously had identical microbial communities to the adjacent field. Generally, stronger relationships existed between PC1 and PC2 (Table 2) and individual ribotypes (Fig. 5) of the different microbial communities with physical properties than chemical properties. Hence, changes in soil texture, moisture, BD, and OM content were important in changing the microbial communities. We found no relationships with microbial factors and detachment processes as assessed through the minimal dispersion fine silt + clay or particulate phosphorus release assays. However, there were indications of changes in the relationships between microbial diversity and nutrient solubility, most notably with bacterial PC1 for dissolved organic nutrients and with fungal PC2 and SRP release. Microbial cycles have been well studied with respect to denitrification in riparian wetlands (e.g., Knowles, 2005). Although we found that bacterial PC1 (Table 2) and TRF175 (Fig. 5) were significantly related to concentrations of NO_3, we did not find consistent results as to changes in NO_3 concentrations between buffers and fields to confirm that nitrification was consistently occurring in the VBS. Studies of

microbial P cycling are rarer, despite the fact it has long been appreciated that microbial biomass P can constitute 2 to 5% (cropland) to 20% (grassland) of soil organic P (Stevenson, 1986). Studies of microbial P cycling in situ (as opposed to batch cultures) are even rarer, and this cap on understanding will limit our ability to manipulate this important pool of P to develop mitigation strategies (Ehlers et al., 2010).

Wider Implications for Buffer Management

Changes in soil microbial structure may be related to changes in soil properties but also to vegetation composition (Kennedy et al., 2005; Schütte et al., 2001; Singh et al., 2009). Figure 2 shows a typical example of the pronounced change in the vegetation community between the field and VBS. Typically, these VBS are unmanaged and left to colonize with a succession of grasses and eventually shrubs and trees. Elevated soil nutrient concentrations in the VBS have implications for vegetation and their biodiversity value. Plants perceived to be of high biodiversity value and indicative of "pristine" habitats are outcompeted by rapidly growing scrub species tolerant of higher nutrient levels. This rapid growth of grasses and other annual vegetation is part of the cycle of plant growth–dieback–organic matter accumulation that is implicated in the greater nutrient release. The annual dieback of vegetation and exudation of readily available recent-photosynthate C forms into the soil is a strong stimulus for microbial cycling rates and has been previously linked to solubility of SRP in buffers in Sweden (Uusi-Kämppä, 2005). Therefore, vegetation management may be part of breaking this cycle. By removing vegetation (perhaps via grazing or cutting and removal) the C priming effect is minimized and there may be some useful offtake via phytoaccumulation of N and P. Although microbes are effective at competing with plants for soil P, the elevated soil solution nutrient concentrations we have observed show that excess nutrients escape the microbial cycling loop to become available for plant uptake or subsequently leached. Enhancing plant uptake of these solubilized nutrients would have dual benefits: by removing pore-water nutrients that would otherwise leach (Lee et al., 2000) and providing a possible loss pathway via vegetation removal. Loss rates of 4 to 15 kg P ha^{-1} yr^{-1} have been documented through biomass removal (Hoffmann et al., 2009). Buffer biomass harvesting is a recommended strategy for agri-environmental schemes in Finland.

Conclusions

Our study has shown that buffer soils, sampled from VBS with typical characteristics as found in the United Kingdom, had increased nutrient solubility when compared with adjacent cropland soils, using laboratory assays with field-moist soils. This shows a potential for increased leaching of nutrients to waters in situ. Consequently, under current design and management regimes, VBS are not fully protecting headwaters susceptible to eutrophication. The increased solubility of SRP in buffer soils is a critical finding since, if this P form is leached to streams, it will have deleterious effects on water quality. However, there are a number of abiotic factors, unexplored by the present study, which may impact on SRP solid–solution partitioning, namely soil P saturation and sorption competition with organic matter. Our observations of empirical links between altered microbial diversity and nutrient release in buffer and field soils should be taken as evidence for a necessity

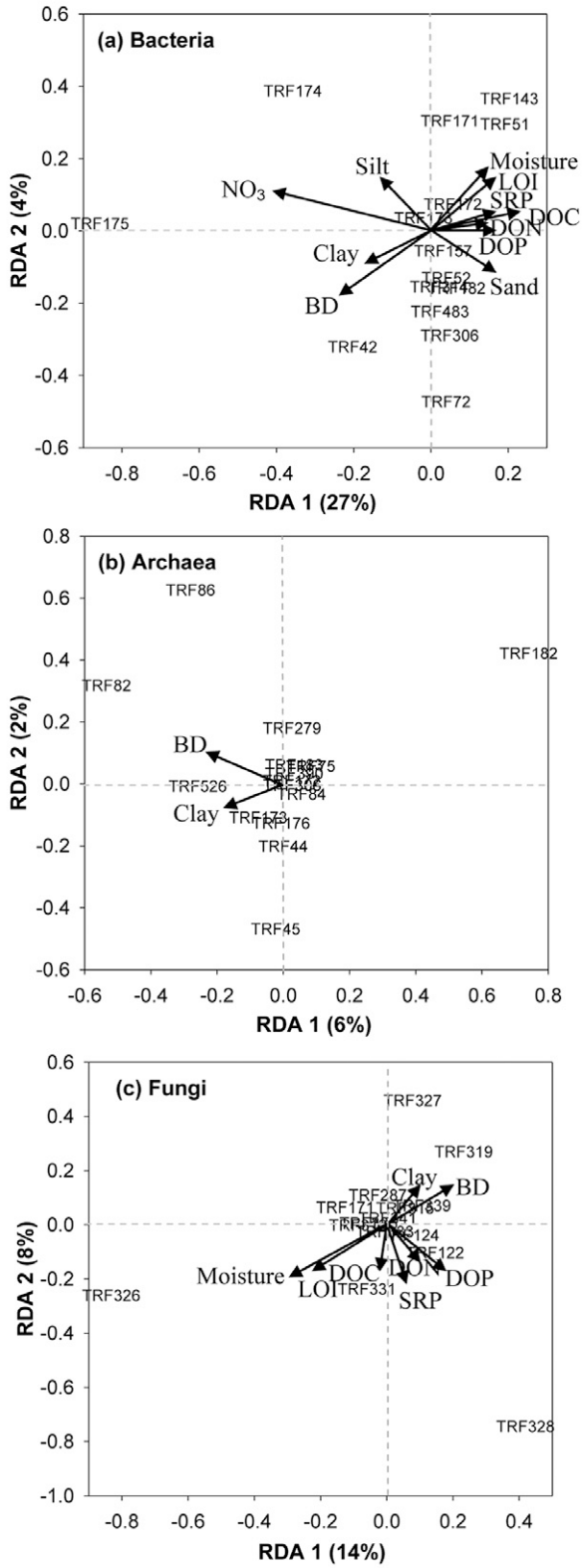

Fig. 5. The response of the 15 most abundant individual terminal restriction fragments (TRFs) from (a) bacterial, (b) archaeal and (c) fungal data sets to significant environmental variables as analyzed by redundancy analyses (RDAs). BD, dry bulk density; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorus; LOI, loss on ignition; OM, organic matter; SRP, soluble reactive phosphorus.

of future mechanistic studies into abiotic and biotic processes. The biotic processes should also be expanded to include the dynamics of the soil–plant–microbial system to understand how to manage processes for the benefit of nutrient losses. The sources of nutrients (particularly P) to buffer soils remain uncertain and could be inputs from upslope (via the erosion retention capacity of the buffer), or solubilization of nutrients remaining from the period of field management and past excess fertilizer application. However, any catchment actions (nutrient source and erosion control measures) acting to limit further increases in nutrient status of buffer soils will decrease the susceptibility that internal nutrient cycling will lead to nutrient solubilization and potentially leaching.

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