Reducing soil fertility to enable ecological restoration: A new method to test the efficacy of Full-Inversion Tillage

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Introduction

It is well known that most semi-natural plant communities of northern Europe require infertile conditions and that when soil conditions become more fertile species diversity reduces and there is an increase in a few dominant species (Grime 1979; Marrs et al. 1996; Critchley et al. 2002; Walker et al., 2004). Where the aim of ecological restoration is to re-establish replacement semi-natural communities there is an implicit requirement that soil fertility must be reduced in some way so that the less-dominant species can establish and maintain themselves (Marrs 1993, 2002). Such ecological restoration is required where soil chemical properties have changes as a result of successional processes (Odum 1971; Gorham et al. 1979), through eutrophication brought about by elevated atmospheric inputs (especially N; Diemont & Heil 1984; Armitage et al. 2012; Britton & Fisher, 2010; Maskell et al. 2010; Smart et al. 2003) and through fertilizer addition (Digby & Kempton 1987; Smith 1988). The residual effect of fertiliser is often found, for example, in restoration schemes on former arable land, where elevated residual P is a particular problem. Various attempts have been made to determine a minimum target values for available P in such restoration schemes and values vary between 10 20 µg P g^{-1}(Walker et al. 2004) and 20 µg P g^{-1} (Gough & Marrs, 1990).

Restoration ecologists have used a variety of strategies to reduce surface soil fertility in restoration schemes, including removal, leaching, sequestration or re-distribution through the profile (but see reviews by Marrs 1993, 2002). The most usual approach is through some form of removal strategy where nutrients are removed usually through the use of continuous cropping - either as grain and straw (Johnston & Poulton 1977) and hay crops (Petgel 1987, Bakker, 1989; Wells 1980 where any effect usually occurs slowly over years or even decades. A more aggressive approach is to remove the topsoil; this has been used a lot in heathland restoration in the Netherlands (Werger et al. 1985; there is a very large and sudden reduction in the total amounts of nutrients in the surface soil, but there may be problems with release of mineralisable N (Dorland et al. 2003).

An alternative approach suggested by Marrs (1993, 2002) was to use some form of deep ploughing to either dilute the concentrated nutrients present in the surface layer through the infertile sub-surface soils so that their effects are reduced or burial where the nutrients are made unavailable at least in the short-term. The burial approach has been pioneered by the charity Landlife in the UK for reducing soil fertility in vegetation improvement schemes (Landlife 2008), although the approach has been used for forestry in Denmark for many years (Landlife 2008). Here, Full-Inversion ploughing (FIV) where a double-bladed plough moves the surface soil underneath the sub-surface layer. The aim is not to reduce the absolute amounts of nutrients on the site; rather they are re-distributed to where they should have a lesser impact on plants rooted in the surface soils. To date, there have been few at-

tempts to test whether this works in potential ecological restoration schemes. Accordingly, here we test the use of a FIV plough on the change in soil chemical properties at two test sites with contrasting soil types, a sandy soil and a clay-loam. We assessed the change in soil chemistry using principal response curves; this approach tests the effects of a given treatment against a control through the soil profile using multivariate soil chemistry data (van den Brink and ter Braak, 1999). This is a somewhat novel approach with respect to the analysis of soil chemistry. Principal response curves are a direct gradient analysis based on a linear distribution model (see van der Brink and ter Braak 1999) that use partial redundancy analysis (pRDA) as a method for ordination. They are almost exclusively used in the assessment of treatment effects on the structure of community matrices through time in repeated-measures experiments (Alday *et al.* 2013, Moser *et al.* 2007).

Methods

Site description

Soil cores were collected from two sites possessing different soil types. The first site was an area of derelict land of ~1 ha located at the University of York, Heslington, Yorkshire, UK (-1.0416290 W longitude; 53.947242 N latitude; National Grid Reference SE 62999 50552). The site was in agricultural use prior to 2011, after which it became ancillary land adjacent to a construction project at the university, and had been used primarily for access to the site, the storage of construction materials and had also suffered heavy disturbance from the laying of service provisions involved in the construction, i.e. drainage and power services. The site has a clay-loam soil.

The second site was an area of semi-suburban green belt grassland of ~1 ha located at Kirby, Merseyside, UK (-2.876170 W longitude; 53.465736 N latitude; National Grid Reference SJ 41702 96915). Presently, the site forms part of a Conservation Area that runs parallel to the M57 motorway but historically was predominantly rural meadowland and willow plantation. The site has a sandy soil.

Soil sampling and analysis

An Eijelkamp auger (12 cm depth, 7.5 cm diameter) was used to collect eight sequentially-stacked semi-disturbed cores of 12 cm length to a depth of 96 cm at locations randomly-selected within each site, both prior to inversion (no tillage - NT) and 42 days after full-inversion tillage (FIT) at the Heslington site or 23 days after FIT at the Kirkby site. Nine cores were taken at Heslington and seven at Kirkby; sampling density was a recommended by (Stolbovoy *et al.* 2005). Each soil sample was placed into an air-tight plastic bag and transferred to a cold-store at the laboratory within three hours of collection. Soil pH m extractable P and NH_4-N and NO_3-N were measured on fresh soil sieved which had been passed through a 2 mm sieve whereas total organic C and N and exchangeable cations were measured on air-dried soil passed through a 1 mm sieve.

Soil pH was measured in a 1:2 (w/v) soil/deionised water mixture using a Hanna Instruments 98103 pH meter (Hanna Instruments, Leighton Buzzard, UK). Soil extractable P concentration was measured using the $NaHCO_3$ extraction method of Olsen (1954) and both available NO_3-N and NH_4-N concentrations were measured using the 2M KCl extraction method of Keeney and Nelson (1987); both followed by colorimetric determination. Exchangeable K, Mg and Ca concentrations were determined by emission/absorption spectrophotometry after extraction in a 1:10 mixture of soil/neutral ammonium acetate at pH 7.0. To measure total C and N soil samples finely-ground in a roller mill; thereafter duplicated sub-samples (ca. 5 mg) were then desiccated and analysed using a Carlo Erba NC2500 series CN Analyser (Carlo Erba Instruments, Milan, Italy). Duplicate replicates that exceeded 0.05 CV were re-analysed.

Statistical analysis

Principal response curves (PRC), testing for a constrained effect of FIT on soil chemistry through the soil profile was performed using the 'prc' function in 'vegan version 2.0-2' (Oksanen *et al*. 2011). Here, the community matrices have been replaced with soil chemistry matrices and the repeated-measures temporal gradient has been replaced by the depth gradient through the soil profile, and so deviations in the soil chemistry through depth in response to FIT have been compared with the soil chemistry through depth in the soil before inversion. A PRC diagram has been used to report the results, with soil depth constrained to the ordinate axis and the principal response (effect) relative of FIT relative to NT on the abscissa. Included in the plots is a complimentary soil chemistry weight which may be interpreted as the weight of each soil chemistry variable for the response reported in the diagram, e.g. a high weight for a given variable would indicate that the response pattern of that variable is more likely to follow the pattern reported in the PRC, or alternatively, low variable weights would indicate a reversal of the PRC (a positive effect of FIT against a negative variable weight is indicative of a decrease in concentration of that variable due to FIT) (van der Brink and ter Braak 1999).

The PRC analyses were performed on Hellinger-transformed soil chemistry matrices for each of the two sites. This approach does not differentially weight soil chemistry variables of low concentration and is appropriate for testing the significance of the relationship between matrix composition and a set of covariates (Legendre and Gallagher 2001). The explanatory power of the models and their goodness of fit were measured by calculating the variance explained (%) by the first canonical PRC axis and its corresponding *eigenvalue* (λ_1), and the associated *F*-ratio and *p*-value determined for significance using Monte Carlo permutations and *F*-tests.

Testing for significant differences between the NT and FIT treatments at each depth strata was performed by applying a Williams Test (Williams 1972) on the first principal component of a PCA at each sampling depth.

Results

Testing for the effects of Full Inversion Treatment on soil chemical properties

For the Heslington site, the PRC revealed that 23.6% of the total variance in soil chemistry was explained by depth through the profile, with 7.5% explained by FIT and its interaction with depth (Fig. 1a). The first canonical axis of the PRC significantly captured approximately 1% of the variance explained by FIT (Monte Carlo permutation test, 144 permutations, $p = 0.004$). From 6 cm to 18 cm depth FIT significantly differed from NT ($p < 0.05$ for both depth strata). There were no significant differences found between FIT and NT for any depths >18cm. The surface soils showed positive weights for all soil variables with the exception of extractable P where there was high negative weight suggesting that the extractable P concentration had been reduced relative to pre-inversion (Fig. 1). Simple t-tests for the individual soil variables in the surface soils shows significant reductions in extractable P and K and increases in exchangeable Ca (Table 1). The exchangeable P concentration had more than halved and was now <10 ($\mu g \ P \ g^{-1}$).

At the Kirkby site, ~10% of the total soil chemistry variance was explained by depth, whilst 24% was explained by the FIT x depth interaction (Fig. 1b). Approximately 0.1% of the constrained variance was significantly captured by the first canonical axis (Monte Carlo permutation test, 112 permutations, $p = 0.001$). Soil chemistry under full inversion tillage was found to differ significantly from NT in the upper 66 cm of the soil profile (p <0.05 for all strata). In the surface soils almost all soil variables were increased after inversion relative to the undisturbed profiles (Table 1). The differences between FIT and the NT were positively associated with weights for exchangeable Ca and extractable NO_3-N, and negatively with extractable P and exchangeable K and Mg (Fig.1)

There were clear differences in the response of the soil chemistry between the two sites; Heslington illustrated a change of the soil profile in terms of soil chemical properties at around 18cm above an infertile layer was produced. This was not apparent at the Kirkby site where the surface soil appeared showed a small increase in most soil variables

Why is there different impacts at the two sites?

One reason there may be differences in treatment effect between sites is the differences in the chemical properties with depth between the two sites. Essentially four different profile types were detected for individual elements: Profile 1, high at the surface and a decline through the profile; Profile 2, low at the surface and increasing down the profile; Profile 3, highest at intermediate depths; and, Profile 4, no change with depth. Examples of the profile types detected at the two sites are presented in Fig. 2. For Heslington, total N and C, exchangeable K, extractable NH_4-N, NO_3-N and P showed Profile 1 (Fig2), soil PH and exchangeable Ca showed profile 2, and C:N ratio and exchangeable Mg showed profile 4. At Kirkby, all but one soil variable exhibited Profile 3, the exception was soil pH with a

profile 4. Essentially, the two sites had different starting profiles, Heslington showing ordered changes with depth and Kirkby showing a curvilinear response; this was almost certainly the reason for the differential effects of the full inversion tillage.

Discussion

In this paper we have demonstrated the potential for the of prc to assess changes brought about by SIT of soils in restoration schemes. The benefits to the use of this approach include (a) a rigorous statistical test of the effects of an intervention treatment relative to an untreated control, (2) a multivariate assessment that identifies the key variables changed by that intervention. We believe this approach has much to offer ecological restoration studies that involve assessment of impacts on soil depth profiles.

Principal Response Curves

One of the difficulties in comparing soil profiles is that the component parts of the profile are spatially autocorrelated and this makes standard statistical approaches such as analysis of variance invalid. Principal response curves in contrast uses a direct gradient analysis based on a linear distribution model (see van der Brink and ter Braak 1999), effectively a regression approach based on a multivariate ordination, partial redundancy analysis (pRDA). Crucially the PRC tested for the effects on an intervention against an untreated control within an experimental control along a gradient, usually a temporal one. Indeed, most analyses using PRC have either been in the field of ecotoxicology testing the effects of a pesticide through time (van der Brink and ter Braak 1999; Moser *et al.* 2007) or in vegetation science (Alday *et al.* 2013). There is no reason why they cannot be used on other gradients and here soil depth is an obvious candidate.

The benefits of this approach are many. First, it is a multivariate test and hence all appropriate variables can be included, here we used a selection of soil variables but this could be extended to included other variables such as mineralization rates or microbial activity. Principal response curves was originally intended for use in formal experimental designs, and its use her does not strictly conform to this as it is a structure survey, there is a treatments comparisons (intervention versus control) down the soil profile gradient. One further extension to this approach could be its use in monitoring soil change through time - we have the baseline undisturbed profile against which to test the intervention, but there is no reason why future data could not be included in further analyses to detect significant change. Essentially this has bene done for vegetation by Alday *et al.* (2013) when testing for the es-

tablishment of alternative stable states in vegetation. We have reproduced generic code to run similar analyses.

The use of Full Inversion Tillage in ecological restoration

It is generally accepted that there is a need for the establishment of infertile conditions for the restoration and maintenance of most semi-natural plant communities in northern Europe (Bakker 1979; Marrs 1993, 2002). This is especially true when attempting to restore such communities on ex-arable soils (Critchley *et al*. 2002; Walker *et al.* 2004) where there may be considerable elevated residual fertility (Marrs 1993). Given this, it is surprising that there have been few attempts to document the use of full inversion tillage in ecological restoration schemes. Landlife, a charity with a mission which *inter alia* includes promoting new wildflower landscapes and creative conservation has pioneered the use of full inversion tillage in a range of conservation schemes throughout the UK, but no attempt to date has made to assess its efficacy. This paper represents the first statistically-rigorous assessment of full inversion tillage

With full inversion tillage, in ideal conditions the soil profile is flipped or inverted with the surface topsoil being placed under what was the previous sub-soil. In most situations the surface topsoil would have a greater fertility than the subsoil and hence almost instantly there should be a reduction in fertility suitable for the establishment of semi-natural plant communities of high conservation value. Here, this result was found for one of the two tests sits (Heslington) where after full inversion tillage there was a significance change in the soil properties of the surface soils to a depth of ca. 24 cm, and the soil extractable P was almost halved in concentration to a level below the 10 μg P g^{-1} threshold identified by Walker *et al*. (2004) as suitable for the maintenance of species-rich grassland. So for this site the results could be viewed as very positive.

The results for the Kirkby were less successful in that there was an increase in most soil variables at the soil surface after full inversion tillage. This can be explained with reference to the soil chemistry of the pre-treatment profile. In contrast to the Heslington site where there was clear directional changes in soil chemical variables with depth for most variables at Kirkby the responses were mainly curvilinear with maximum concentrations in mid-profile. This profile distribution is probably a result of previous fertilisation and substantive leaching through its sandy substrate. When this type of profile is inverted some of the soil chemicals previously found in mid-profile are re-distributed through the surface layers, effectively enhancing the fertility of the topsoil layer. In these situations full inversion tillage is not appropriate for ecological restoration purposes.

Thus, full inversion tillage has considerable potential in ecological restoration schemes where there is a management requirement to reduce surface soil fertility. It reduced the fertility quickly and it is rela-

tively quick to apply as approximately 2-3 ha can be treated in one day (Landlife 2008), although exact area will depend of soil type. However, it is suggested that an ecological impact assessment be carried out before treatment to ensure that the soil profile type is suitable for this treatment (Heslington here but not Kirkby). This should be carried out using the method outlined here – 7 depth samples per ha (Stolbovoy *et al.* 2005). Another situation where fill inversion tillage was considered was to assist with restoration of acid grassland and heathland on ex-arable land at Minsmere in Suffolk in the 1990s (R. H. Marrs, pers. comm.). At Minsmere, there was a need to reduce soil pH, exchangeable Ca and extractable P, but full inversion tillage was deemed inappropriate because there was no significant change in any of these soil properties within a 1 m depth profile (Marrs *et al.* 1998). Once the decision is made to use full inversion tillage then its impact can be tested statistically using the method outlined here and monitored through time if required.
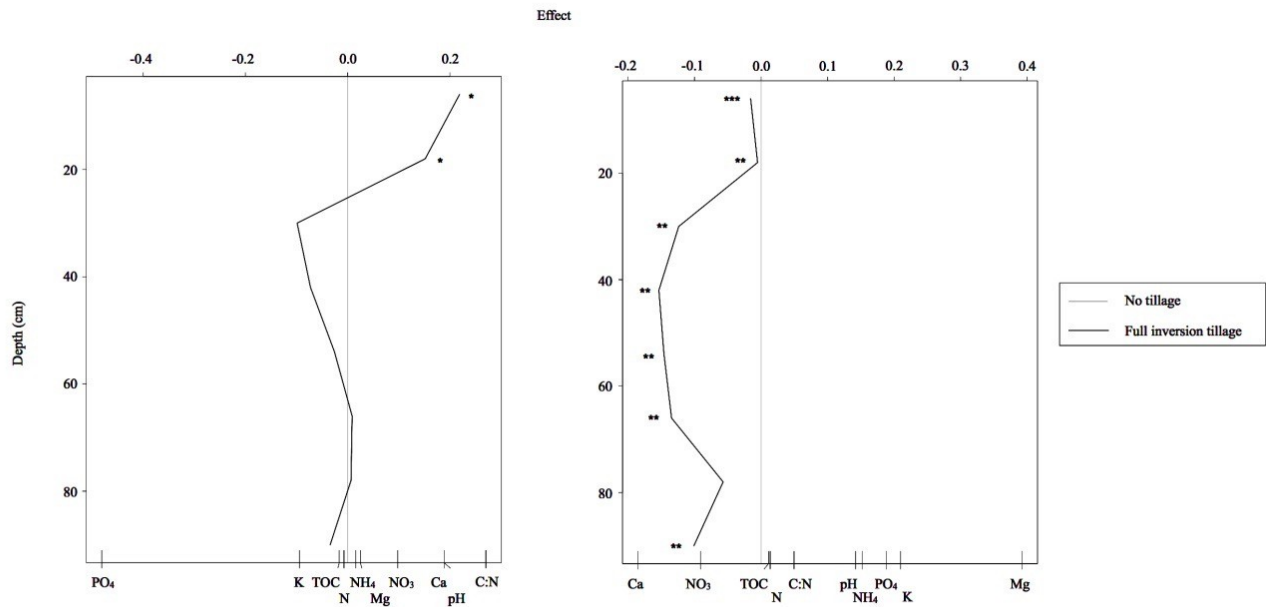
Acknowledgements

Fig. 1. Principal response curves analysis of changes in the soil chemistry down the profile comparing the effects of full inversion tillage to an untreated control in two contrasting sites undergoing restoration. Significant differences (shaded) are denoted; * = P<0.05;**=P<0.01; ***=P<0.001.

Table 1. Surface soil chemical properties (0-12 cm) in undisturbed and inverted profiles at two contrasting sites; mean values sites ±SE and t-values are presented. Significant differences (shaded) are denoted; * = P<0.05; ***=P<0.001.

| Soil variable	Kirkby (=7)			Heslington (n=9)		
	Undisturbed	Inverted	t	Undisturbed	Inverted	t
pH	6.1±0.06	6.1±0.1	0.8	7.2±0.14	7.4±0.1	1.4
Total C (%)	0.7±0.2	3.2±0.1	13.8***	1.4±0.2	1.0±0.1	2.0
Total N (%)	0.06±0.01	0.22±0.03	6.2***	0.13±0.02	0.08±0.01	3.0
C:N	11.6±1.01	15.3±1.34	2.6*	11.0±0.52	12.5±0.8	1.7
Available P (μg P g^{-1})	2.9±0.7	11.7±1.2	7.6***	14.8±2.2	6.7±2.6	2.7*
Available NH_4–N (μg N g^{-1})	1.6±0.7	6.0±1.6	2.9*	2.7±1.7	2.4±1.3	0.2
Available NO_3-N (μg N g^{-1})	1.3±0.6	7.7±1.1	6.1***	2.3±0.6	2.3±0.4	0.1
Exchangeable K(μg K g^{-1})	24.6±4.3	138.8±16.0	8.2***	96.7±12.2	68.4±6.4	2.3*
Exchangeable. Ca(μg Ca g^{-1})	530±99	1708±75	11.2***	1.8±0.17	2.8±0.4	2.5*
Exchangeable Mg (μg Mg g^{-1})	26.5±6.7	65.5±7.1	4.7***	0.1±0.01	0.1±0.03	0.4
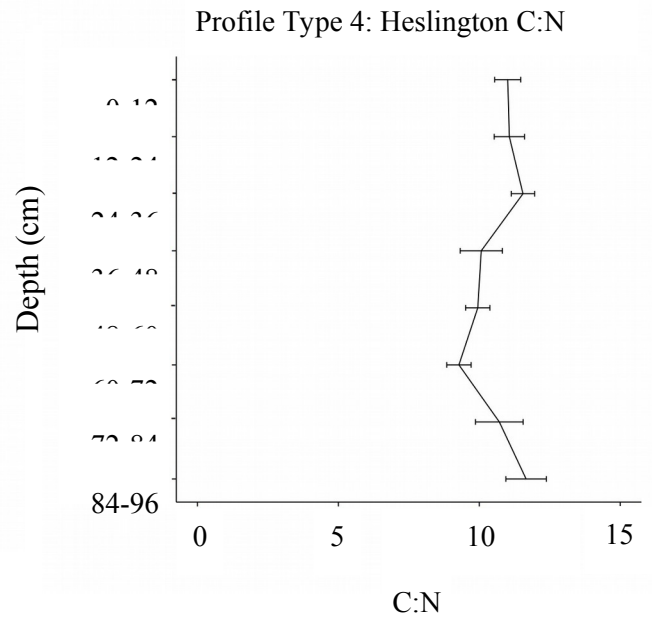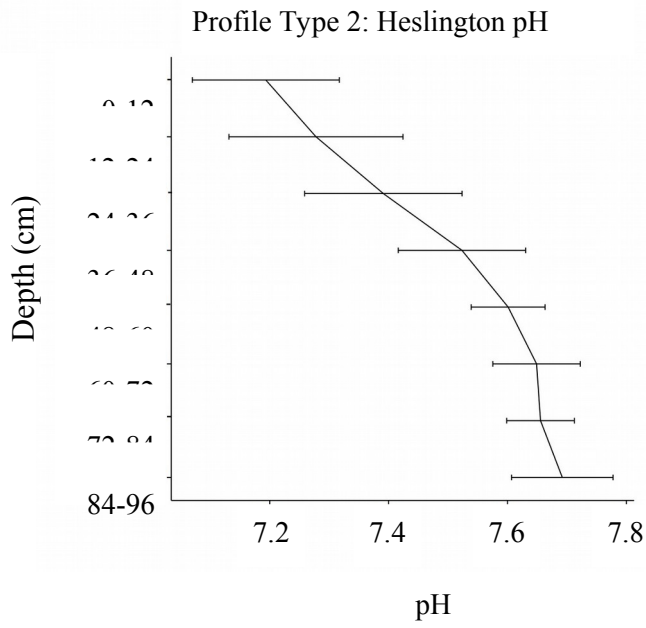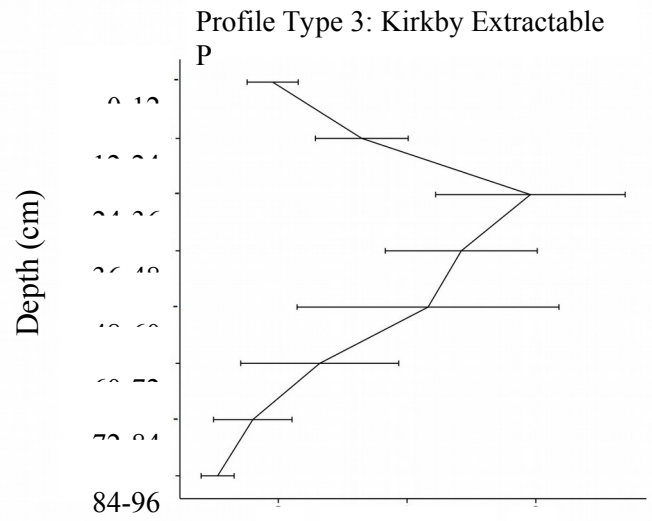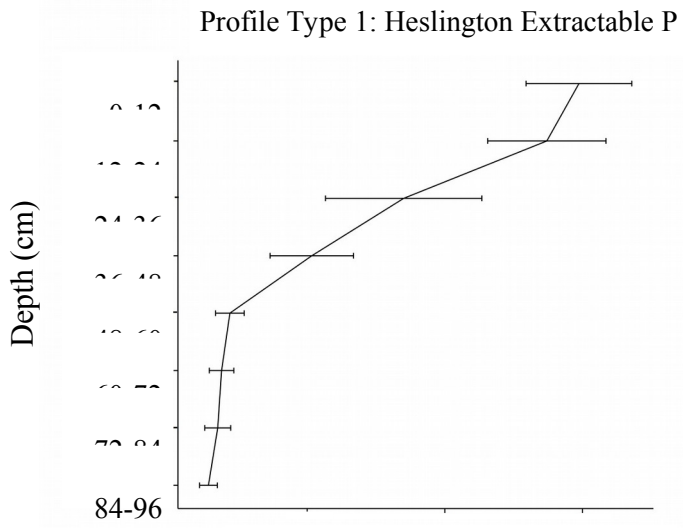
Fig. 2. Four different examples of undisturbed soil depth profiles detected at the two test sites.

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