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Functional stability, substrate utilisation and biological indicators of soils following environmental impacts

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Abstract

Stability of a soil property to perturbation comprises both resistance and resilience. Resistance is defined as the ability of the soil to withstand the immediate effects of perturbation, and resilience the ability of the soil to recover from perturbation. Functional stability is used here to describe the stability of a biological function to perturbation, rather than the stability of physical structure or chemical properties. The function chosen for this study was the short-term decomposition of added plant residues, and the perturbations were copper and heat stresses. Previous studies had shown that functional stability was reduced greatly in soils with experimentally reduced biodiversity. The objective of this study was to determine the relative sensitivity of functional stability and potential indicators of biological status to detect alteration of field soils by various environmental impacts. Functional stability, protozoan populations and substrate mineralisation kinetics, were measured on paired soils with: high or low plant species diversity; hydrocarbon pollution or not; extensive or intensive agricultural management practices. Substrate mineralisation kinetics were poorly related to the soil's antecedent conditions and were stimulated significantly by hydrocarbon pollution. Protozoan populations were potentially useful for detecting differences within soil type, but will require greater taxonomic input to be most useful. Functional stability, particularly resistance, was able to quantify differences between and within soils. The potential development of the technique in relation to soil health is discussed. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Soil resilience and stability are part of the wider concepts of soil health and quality, which are used to describe the overall state of a soil. Definitions of soil health and quality overlap to a major degree, but soil quality focuses more on the soil's capacity to meet defined human needs whilst soil health focuses more on the soil's continued capacity to maintain its functions (Pankhurst et al., 1997). A current challenge is to maintain agricultural productivity whilst using the natural resilience of soils to establish sustainable production in unimproved and degraded systems, in the face of continued population increase (Greenland and Szabolcs, 1994). Soil resilience is a concept embracing many aspects, and a simplified definition, covering the most important, is that it is 'the soil's ability to recover after disturbance' (Greenland and

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Szabolcs, 1994). The response of soils and ecosystems in general to disturbance has two components, resistance and resilience, whose combined effects determine what ecologists refer to as ecosystem stability. Resistance is the inherent capacity of the system to withstand disturbance, whereas resilience is the capacity to recover after disturbance (Pimm, 1984; McNaughton, 1994; Seybold et al., 1999).

Although the conceptual basis of resilience is well defined, there are difficulties in quantification. Seybold et al. (1999) quoted a list of 14 potential indicators of soil resilience, covering a broad spectrum of soil physical, chemical and biological characteristics. Szabolcs (1994) and Lal (1994) both attempted to describe soil resilience using formal equations, but incorporating qualitative factors such as: antecedent soil condition, external management inputs, biological buffering, and anthropological soil fluxes. However, these are essentially conceptual descriptions rather than precise measures. Biodiversity is a soil property important for the soil's capacity to recover from perturbations (Pankhurst et al., 1997). Maintenance of the biological status of soil is generally regarded as a key feature of sustainable production (Swift, 1994), to ensure ecosystem functions such as decomposition, nutrient cycling, and soil structural genesis. Even if high species richness does not always play a significant role in maintaining ecosystem processes under normal environmental conditions, it may be important when conditions change (Yachi and Loreau, 1999). There is evidence for this diversity-stability relationship in terrestrial ecosystems (King and Pimm, 1983; Frank and McNaughton, 1991; Tilman and Downing, 1994; Sankaran and McNaughton, 1999) as well as in aquatic microcosms (McGrady-Steed et al., 1997; Naeem and Li, 1997). However, it is not known how much biodiversity is needed to ensure continuance of specific soil functions (Pankhurst et al., 1997).

The biodiversity of microbial communities is such that there is a degree of redundancy in the species present and, therefore, a generally high degree of resilience in biological functions (Swift, 1994; Finlay et al., 1997). Biological indicators (species composition, biomass or biodiversity) cannot generally be used to quantify soil health (Pankhurst et al., 1997). Dighton (1997) recognised that only by using functional assays, which integrate both microbial community structure and species composition, could the functional aspects of the microbial community be assessed. The effect of experimentally reducing biodiversity of a soil can have contrasting effects on different functions, as predicted by Pankhurst (1997). In a recent study, as biodiversity was reduced, nitrification decreased while decomposition increased (Griffiths et al., 2000). In contrast to individual functions, there was a direct link between biodiversity and stability in those soils with experimentally reduced biodiversity. As biodiversity declined, decomposition became less stable (i.e. both less resistant and less resilient) to experimental perturbations (Griffiths et al., 2000).

Another functional approach to assessing soil biological status is to analyse the mineralisation kinetics of different substrates. The decomposition of a range of substrates added to soil is multiphasic, and this occurs when a single population with multiple uptake systems or where more than one population of microbe capable of mineralising the compound is present (Schmidt and Gier, 1990). Hu and van Bruggen (1997) demonstrated that the multiphasic decomposition of cellulose is controlled interactively by C and N availability and the structure of the microbial community. Thus, given similar nutrient status, the pattern of substrate decomposition should reflect microbial community structure.

In a previous experimental demonstration of functional stability, soil was treated harshly to reduce biodiversity (fumigation with chloroform vapour for up to 24 h, Griffiths et al., 2000). It was possible that the experimental levels of biodiversity reached were unrealistically low compared to naturally prevailing levels in soils. Thus, the assay of soil functional stability might not be sensitive to changes in biodiversity such as may occur in the field. The objective of the study reported here was to determine functional stability and mineralisation kinetics of field soils that were expected to differ in biodiversity a priori. The intention was to confirm whether laboratory assays of functional stability and mineralisation kinetics can be sensitive to changes in biodiversity of natural as well as experimental soils. Six soils were chosen for study from three paired sources: a model ecosystem experiment where soils had been planted with either a grass monoculture or a mixture of six grass species; an organically farmed field and a field under intensive vegetable production; soil contaminated with hydrocarbons and an uncontaminated control soil. This gave

a wide range of environmental impacts varying from a small increase in plant biodiversity, through contrasting management regimes, to a polluted industrial situation, and thus a range of expected differences in biodiversity.

2. Materials and methods

2.1. Soils

The soils were chosen with the a priori assumption that they would differ in biodiversity. Soils came from three sites:

1. from model ecosystems constructed from intact monoliths (0.71 m × 0.71 m × 0.28 m) excavated from an old field on the CNRS campus, Montpellier, France as described by Dhillion et al. (1996). This was a clay-loam soil with <2.5% organic matter and a pH of 8.2. Soils for this experiment were collected after the growing season from treatments planted 5 years previously with either a single annual grass species, or six annual grass species typical of Mediterranean old-fields. Samples taken during the growing season of the first year had shown that the microbial community in the soil with six species was functionally more diverse than that from the soil with the monoculture (Dhillion and Roy, unpublished data). These two soils are subsequently referred to as the one-species and six-species grassland soils.
2. from a polluted site (formerly a petrol station) undergoing remediation, in Göttingen, Germany. Both the polluted and the control uncontaminated soil were taken from a depth of ca. 3 m, being the B-horizon of a sandy-loam soil. It was anticipated that the lack of plant-derived inputs would have led to an impoverished microbial community and that the polluted soil would, intuitively, have an even more impoverished microbial community than the uncontaminated site. The polluted soil contained a variety of petroleum products, the most abundant of which were: benzene (45 mg kg^{-1} dry weight), toluene (320 mg kg^{-1} dry weight), ethylbenzene (180 mg kg^{-1} dry weight), xylene (510 mg kg^{-1} dry weight), and trimethylbenzene (510 mg kg^{-1} dry weight). These two soils are subsequently referred to as industrial soils.
3. from intensively and extensively managed horticultural farms from the Eden valley, Fife, UK. The intensively managed soil was a freely drained loamy sand, developed on fluvioglacial sands-and-gravels derived from upper Old Red Sandstone sediments. Intensive, in this case, means the use of inorganic fertilisers, herbicides and biocides, no organic matter additions, and multiple cropping. The extensively managed soil was a freely drained sandy-loam, developed on till derived from upper Old Red Sandstone sediments. Extensive, in this case, is organic farming under the guidelines of the Soil Association, with no inorganic fertilisers, herbicides and biocides, and frequent organic matter additions. It was anticipated that the organically managed soil would have a greater biodiversity than the intensively managed soil. Both soils were collected from the upper 10 cm during the growing season, and are subsequently referred to as the agricultural soils. All soils were stored moist at 15°C prior to use.

Initial studies were carried out using the grassland and industrial soils. These indicated that substrate utilisation techniques as described below (both community-level physiological profile (CLPP) and mineralisation kinetics) did not reveal differences in biological status. Thus, subsequent studies with the agricultural soils did not examine substrate utilisation but concentrated on functional stability.

2.2. Biological indicators

Background information on the biology of the different soils was obtained by determining: protozoan populations and the mineralisation of C from added plant residues (all soils), and additionally basal respiration and the CLPP of the soil bacteria (grassland and industrial soils only).

Protozoa were enumerated from a suspension of 10 g wet weight soil in 40 ml sterile Neff's modified amoeba saline (NMAS; Page, 1976) by a most-probable-number technique (Darbyshire et al., 1974). 4 × 0.1 ml aliquots of the suspension were serially diluted threefold in 1/10th strength nutrient broth (Oxoid) in NMAS, and microscopically checked for protozoa (flagellates, ciliates and naked amoebae) after 7, 14 and 21 days incubation at 15°C. Protozoan biomass was calculated assuming an average

size of 50 μm^3 per flagellate, 400 μm^3 per amoeba and 3000 μm^3 per ciliate (Stout and Heal, 1967) and a dry weight conversion factor of 0.212 pg μm^{-3} (Griffiths and Ritz, 1988). Total protozoan biomass was the sum of the three protozoan groups. Three replicate soil suspensions were prepared for each soil.

The CLPP (Garland and Mills, 1991) was determined using soil Biolog ECO plates (Biolog, Hayward, CA, USA). Sufficient soil suspension was diluted with 25 ml NMAS to give an absorbance of 0.4 at a wavelength of 595 nm, and 150 μl inoculated into each well. The plates were incubated at 15°C and the absorbance of each well at 595 nm was measured daily with an automatic plate reader for 5 days. The time-course profiles of the Biolog data were analysed from the area under the colour development profile (Hackett and Griffiths, 1997). The Biolog ECO-plates are a modification of the Biolog-GN plates (Insam, 1997), which have three replicated blocks of 31 substrates and a control, rather than 95 individual substrates and a control. The three replicate substrates on each plate enabled for a comparison of the utilisation of individual substrates between treatments by ANOVA. The 93 areas-under-the-curve (i.e. three replicates of 31 different substrates in each plate) were also analysed using principal component analysis with GENSTAT v. 5 release 3.2 (Payne et al., 1993) to distinguish between treatments. The number of positive wells, i.e. those with an optical density greater than 1.4 times the control well (which has no added carbon substrate), on each day was analysed using analysis of variance. Mineralisation of C from the soils (basal respiration) and from added ryegrass (*Lolium perenne* L.) residues were determined as part of the functional stability and mineralisation kinetics assays described below.

2.3. Mineralisation kinetics

The extent to which the respiration response to added carbon was limited by the availability of nitrogen and phosphorus was determined. Aliquots of 3 g dry weight equivalent of soil were mixed with glucose (3.2 mg C g^{-1}), then amended with solutions of $(NH_4)_2SO_4$ or KH_2PO_4 to give a C:N:P of 10:2:1. All combinations of C, N and P were used to determine soil nutrient limitations. Final gravimetric moisture content was 40%. The amended soils were incubated at 20°C in an automated electrolytic microrespirometer, as described by Scheu (1992), and oxygen consumption was recorded at 30 min intervals for up to 3 days.

Subsequently soils were mixed with powdered substrates to give 3.2 mg C g^{-1} dry soil of: glucose, *L. perenne* shoot material (C:N 9), mixed species sawdust (C:N 808), carboxy-methyl cellulose (only added to the industrial soils, not the grassland soils), or a control (no added substrate). Substrates were added to 6 g dry weight soil, apart from glucose which was added to 3 g dry weight soil otherwise the oxygen consumption was too large to be recorded. After adding the substrate, the soils were amended with solutions of $(NH_4)_2SO_4$ and KH_2PO_4 to give a C:N:P of 10:2:1 (as the initial experiment had shown nutrient limitation), and oxygen consumption recorded as above.

2.4. Functional stability

This assay was performed essentially as described by Griffiths et al. (2000). Soils were divided into 10 g aliquots and randomly allocated to the stability treatments. The aliquots were: untreated (control); amended with copper (powdered $CuSO_4$ to give 500 μg Cu g^{-1} dry soil); heated (40°C for 18 h in a sealed container); or frozen (−20°C for 18 h in a sealed container, agricultural soils only). After treatment the aliquots were incubated at 15°C and watered regularly with sterile distilled H_2O to maintain a constant weight. At intervals (ca. 1 day, 2 weeks and 2 months) after treatment three replicate aliquots were removed and the mineralisation of C from added *L. perenne* shoot material (same substrate as used for the mineralisation kinetics) determined. Enough shoot material to give 3.2 mg C g^{-1} dry soil was mixed into the aliquot of soil (additional N and P was not added for this assay, the C:N of the material was sufficient to offset nutrient limitation). Oxygen uptake by the grassland and industrial soils over 24 h was determined by microrespirometry, as above, while CO_2 evolution over 24 h from the agricultural soils was determined by gas chromatography (Ritz et al., 1992). Due to the absolute differences in respiration rate between soils, resistance was normalised to the control value, i.e.

$$\text{Resistance} = \%\,\text{change from control} = \left(\text{control CO}_2 - \frac{\text{treated CO}_2}{\text{control CO}_2}\right) \times 100\%$$

The statistical significance of the resistance was calculated by bootstrapping. Resilience was taken to be the change in resistance over time.

Fungal growth on the powdered ryegrass that had developed on the industrial soils during incubation was recorded by photography. The undisturbed upper surface of the soils, still in the microrespirometer cuvettes, were visualised using a dissecting microscope with incident illumination.

3. Results

3.1. Biological indicators

The protozoan populations in the two grassland soils were indistinguishable (Table 1). Protozoan biomass in both industrial soils was very low, and there were significantly ($p<0.001$) fewer flagellates in the polluted than the uncontaminated soil. The organically managed agricultural soil had the largest protozoan biomass of all the soils tested, and contained significantly ($p<0.05$) more amoebae than the intensively managed agricultural soil (Table 1). When the CLPPs were analysed, there were no significant differences in the number of substrates utilised or the overall pattern of utilisation between the two grassland soils, or between the two industrial soils. Principal components 1 and 2 of the area-under-the-curve data accounted for 61 and 14% of the variation, respectively, in the grassland soils, and 51 and 32%, respectively, in the industrial soils, but there were no significant differences between the PC scores of the two grassland soils or the two industrial soils. When individual substrates were compared there were differences with only one substrate between the grassland soils, glycyl-L-glutamic acid being utilised to a significantly ($p<0.05$) greater extent in the one-species grassland soils than the six-species grassland soils. There were differences in the utilisation of three substrates between the industrial soils, L-arginine, itaconic acid and phenylalkylamine were utilised to a significantly ($p<0.05$) greater extent by the polluted soil than the uncontaminated soil.

Table 1
Protozoan biomass (ng g^{-1}) of the soils used in this study (n=3)

Soil	Amoebae	Flagellates	Ciliates	Total biomass
Grassland				
One species	406	42.1	12.8	485
Six species	446	43.4	11.9	509
S.E.D[a]	0.06	0.32	1.10	0.08
Industrial				
Uncontaminated	8.9	7.7	2.5	21.1
Polluted	4.0	0.05***	2.5	6.8
S.E.D.	0.77	0.26	–	0.46
Agricultural				
Organic	900	600	n.d.[b]	1400
Intensive	200*	250	n.d.	500*
S.E.D.	0.65	0.53	–	0.35

[a] Standard error of the difference of the mean. Data were $\log_e$ transformed to equalise variances. S.E.D. of transformed data and detransformed means shown in table.

[b] n.d. denotes non-detected.

* Biomasses of the two soils within the same site significantly different at $p<0.05$.

*** Biomasses of the two soils within the same site significantly different at $p<0.001$.

3.2. Mineralisation kinetics

The respiration of glucose in the grassland soils was limited by the availability of N and P (Fig. 1A), but the decomposition of the substrates in soil amended with N and P was not significantly different between the two grassland soils (Fig. 1B–E). Basal respiration exhibited an initial flush of activity within the first few hours of setting the apparatus up, but was stable thereafter. Grass elicited the most rapid response of all the substrates with a first peak after ca. 10 h and a second maximum after ca. 30 h. Peak respiration after glucose addition was after ca. 24 h and was the largest value of all the substrates, while sawdust induced a slow, small rise in respiration in the latter third of the incubation. Respiration in the industrial soils was also limited by the availability of N and P (Fig. 2A), but there were significant differences in the utilisation of all the substrates between the polluted and the uncontaminated soils (Fig. 2B–F). In all cases the polluted soil utilised the substrates more rapidly than the uncontaminated soils. Basal respiration, and respiration induced by grass and glucose behaved as in the grass-

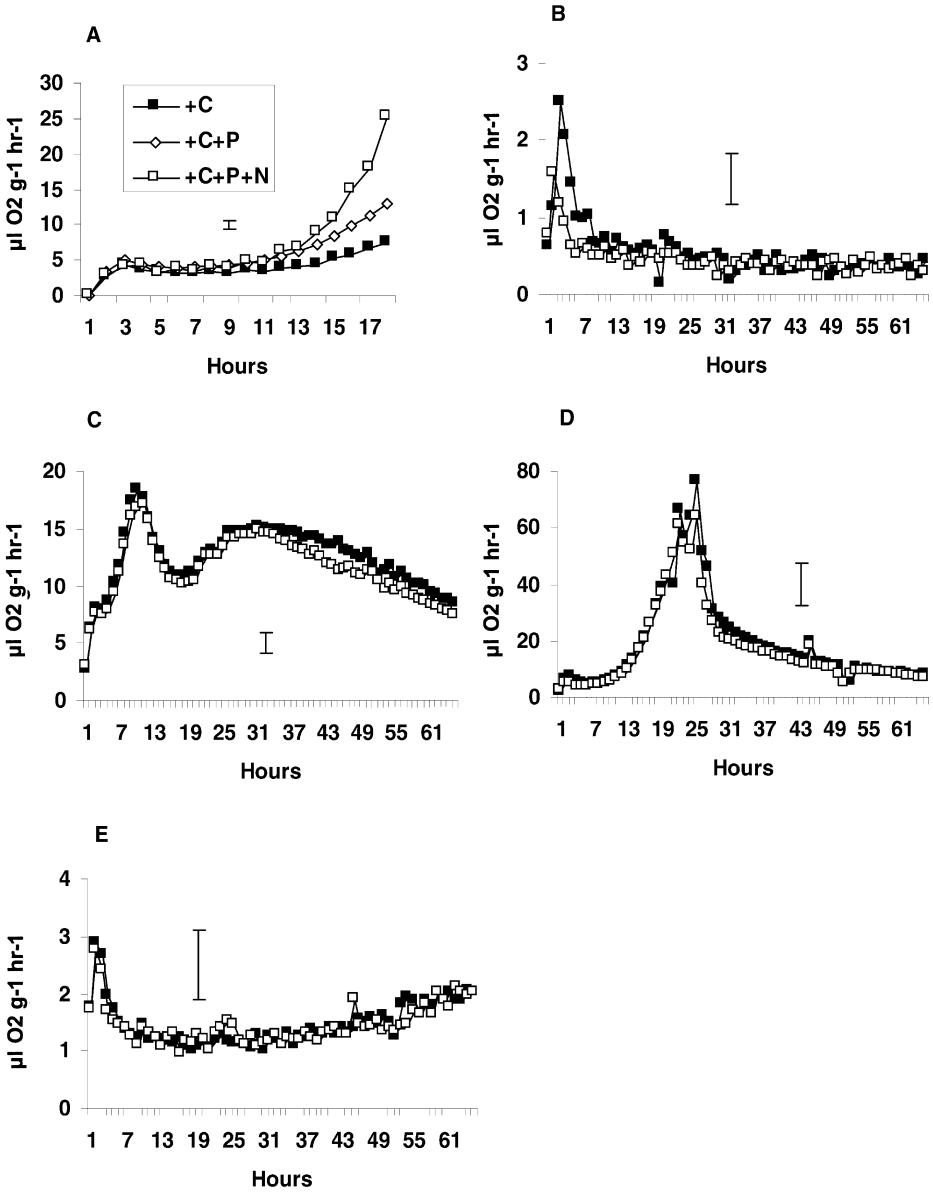


Fig. 1. Respiration (µl O_2 g^{-1} dry soil h^{-1}) from the six-species grassland soil amended with C, N and/or P (A), and from the one-species (□) and six-species (■) grassland soils unamended (B) and amended with grass (C), glucose (D) or sawdust (E). Bar represents greatest S.E., n=3.

land soils. Respiration following the addition of sawdust peaked early, after ca. 15 and 30 h for the polluted and uncontaminated soils, respectively, and then declined gradually. Respiration following the addition of cellulose to the polluted industrial soil was similar to that following the addition of sawdust to the grassland soils, rising slowly in the latter third of the incubation. The uncontaminated industrial soil, however, did not respond to the addition of cellulose within the 3-day incubation.

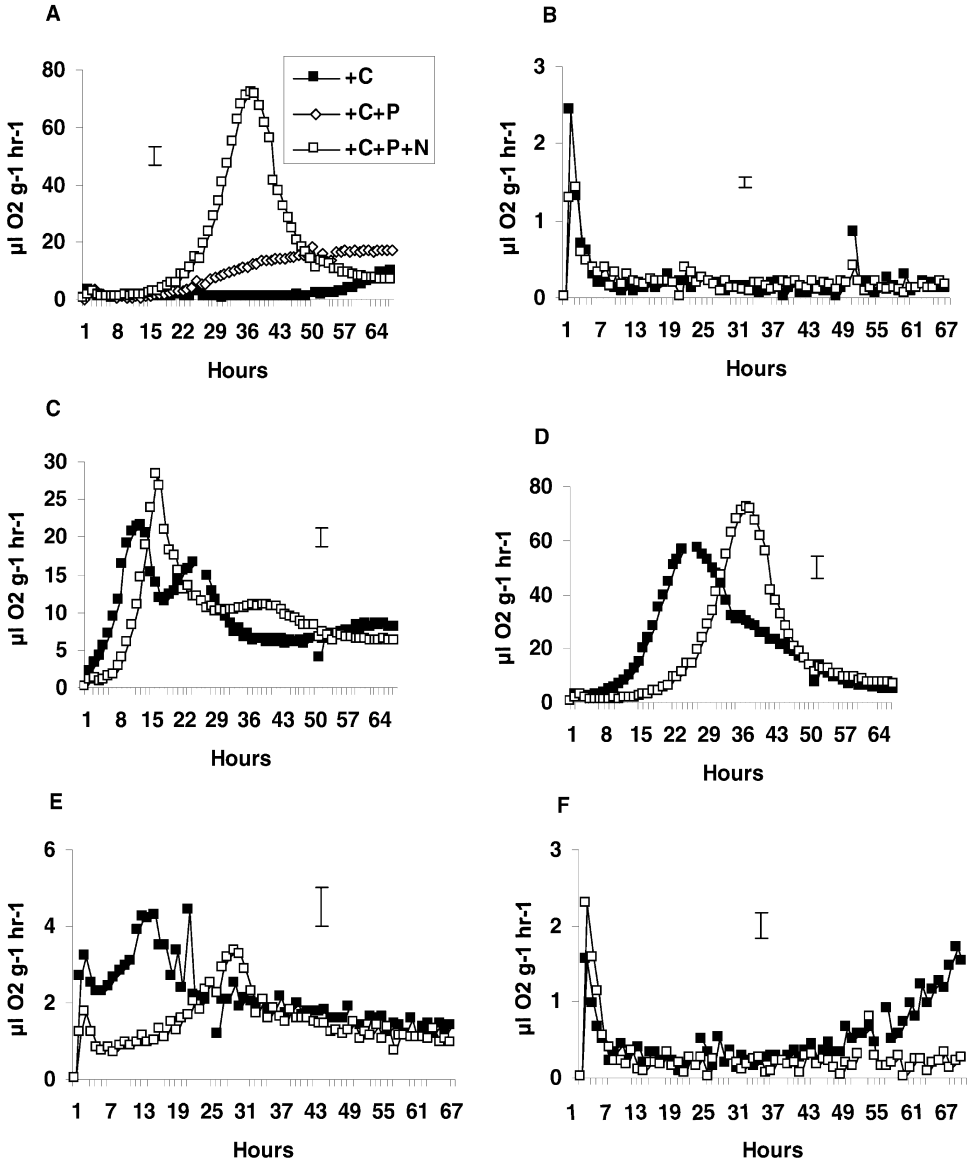


Fig. 2. Respiration (µl O_2 g^{-1} dry soil h^{-1}) from the uncontaminated industrial soil amended with C, N and/or P (A), and from the uncontaminated (□) and polluted (■) industrial soils unamended (B) and amended with grass (C), glucose (D), sawdust (E) or cellulose (F). Bar represents greatest S.E., n=3.

3.3. Functional stability

The time-course of respiration following the addition of grass to stressed soils showed that copper reduced the size of the first respiration peak in both the grassland and industrial soils (Fig. 3A), while the heat stress had a marked effect only in the industrial soils (Fig. 3B). Examination of the industrial soils with a dissecting microscope after incubation revealed differences in the fungal population due to soil type (uncontaminated and polluted) and stress (copper and heat). There was extensive growth of fungal hyphae on the added grass in the non-stressed and copper-stressed uncontaminated soils (Fig. 4A and E), but predomi-

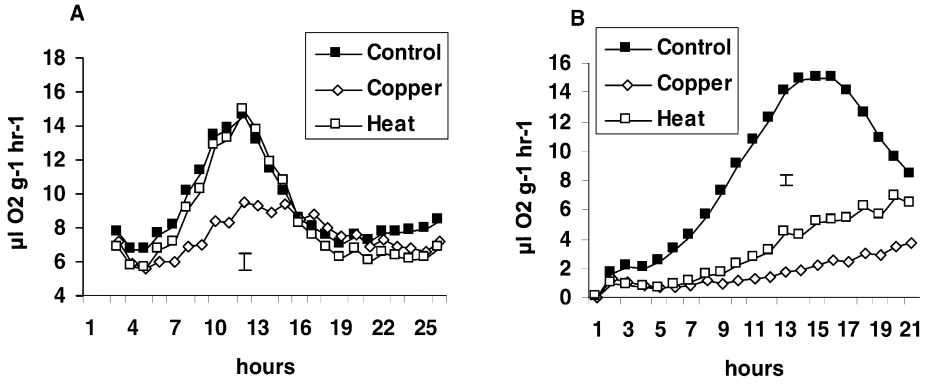


Fig. 3. Respiration (μl O_2 g^{-1} dry soil h^{-1}) from grass added to the six-species grassland soil (A) and the uncontaminated industrial soil (B) that had been unstressed or stressed with copper or heat. Bar represents greatest S.E., n=3.

nantly mould-like colonies without extensive hyphae in the heat-stressed uncontaminated soil (Fig. 4C). There was no detectable fungal growth in any of the polluted soils (Fig. 4B, D and F).

The cumulative respiration in the 24 h following the addition of grass did not differ significantly between the two grassland soils (72.5 μl O_2 g^{-1}±1.3 S.E. and 72.7±1.9 for the one and six species soils, respectively) or the two industrial soils (70.0 μl O_2 g^{-1}±2.8 S.E. and 67.4±1.4 for the uncontaminated and polluted soils, respectively). Respiration from grass added to the organically managed agricultural soil (368.1 μl CO_2 g^{-1}±6.8 S.E.) was greater than that from the intensively managed agricultural soil (309.7 μl CO_2 g^{-1}±6.1 S.E.).

In terms of resistance, soils could be grouped into three clusters (Table 2). (i) Both grassland soils and the organically managed agricultural soil, which had the greatest resistance to both copper (16–20% reduction in decomposition) and heat (7–10% reduction). (ii) The intensively managed agricultural soil, which had intermediate resistance to copper (57% reduction) and heat (25% reduction). (iii) Both industrial soils, which had the least resistance to copper (73–77% reduction) and heat (58–85% reduction). Resistance to freezing was only tested on the agricultural soils, and while freezing stimulated decomposition the effect was greater for the intensively managed (decomposition was 112.84%±2.09 S.E. that of the control soil) than the organically managed soil (101.35%±4.63 S.E.).

The soils could not be grouped as readily in terms of resilience as they could for resistance (Table 2). After adding copper both the grassland soils showed no recovery over 2 weeks, but the one-species soil did recover the decomposition function over 2 months. Two months after copper addition decomposition in the one-species soil had 'overshot' the control soil value. The polluted industrial soil showed a marked recovery after the addition of copper, with decomposition activity stabilising at ca. 20% less than the control soil. The uncontaminated industrial soil showed some recovery after 2 months, and decomposition activity was affected to a significantly greater extent than the polluted soil after 2 weeks and 2 months. The agricultural soils showed no recovery following the addition of copper. After the heat stress the six-species grassland soil recovered decomposition activity to the level of the control soil after 2 months. Both the industrial soils recovered some degree of decomposition activity after 2 weeks, with activity stabilising at ca. 20 and 60% less than the control soils for the uncontaminated and polluted soils, respectively. Decomposition activity in the organically managed agricultural soil was not significantly affected by heat. The intensively managed agricultural soil recovered the majority of its decomposition activity 2 weeks after the heat stress, stabilising at ca. 7% less than the control soil. Following the freezing stress (applied to the agricultural soils only), decomposition activity in the intensively managed soil had recovered to that of the control soil after 2 weeks (103.15%±3.2 S.E.). Decomposition activ-

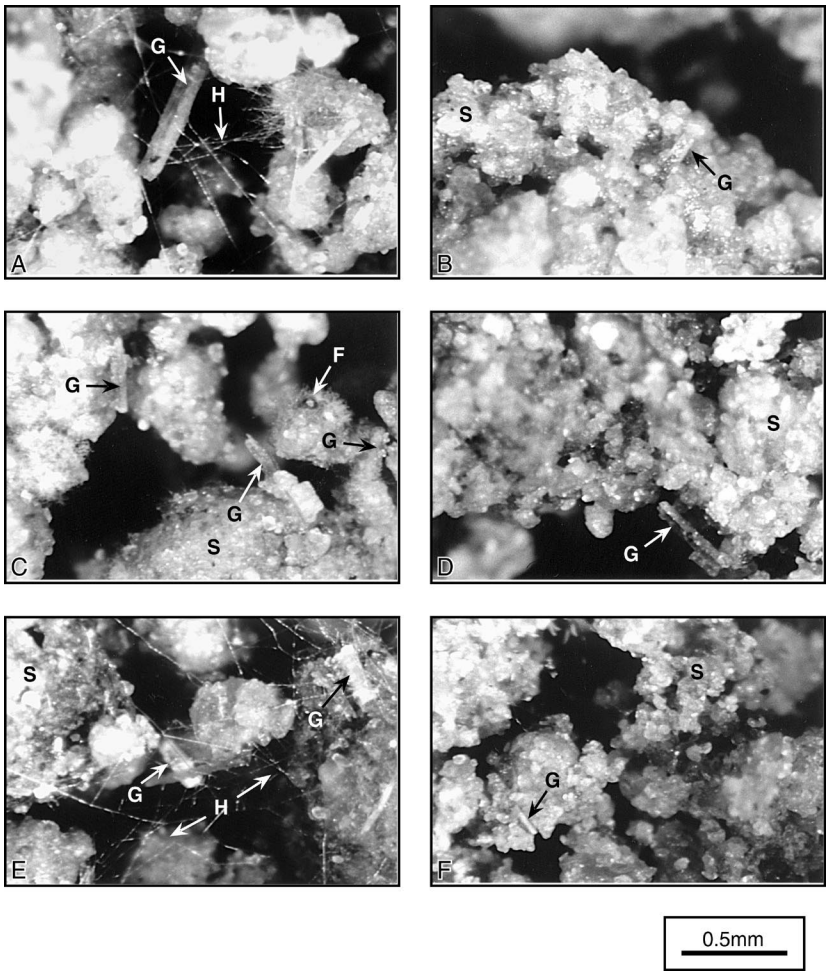


Fig. 4. Photographs of the industrial soil after incubation with grass residues for 72 h at 20°C. The uncontaminated soils (A, C and E) and polluted soils (B, D and F) were left untreated (A and B), or stressed with heat (C and D) or copper (E and F). Photographs show soil particles (S), grass residues (G), fungal hyphae (H) and mould-like fungal colonies without visible hyphae (F). Scale bar represents 500 μm.

ity in the organically managed soil was unaffected by freezing at any time.

4. Discussion

The CLPP analysis could only detect minimal differences within the grassland and industrial soils. Most published studies have used the Biolog GN plates which contain a larger selection of test substrates (95) than the ECO plates (32). Choi and Dobbs (1999) showed that the ECO plates were equally as effective as the GN plates in discriminating between microbial communities. Most CLPP studies have been able to detect changes in microbial community structure due to applied stresses such as: zinc (Kelly et al., 1999a); flooding (Bossio and Scow, 1995) and herbicides (Engelen et al., 1998; El Fantrousi et al., 1999). But stresses did not necessarily lead to a distinguishable CLPP pattern (e.g. in response to heavy metals, Knight et al., 1997; Kelly et al., 1999b). CLPP can also distinguish soils amended with crop residues (Sharma et al., 1999), substrate additions (Hodge et al., 1999) and between the rhizospheres of different plants (Grayston et al., 1998). It is difficult to say whether changes in CLPP pattern represent a consequential

Table 2
Short-term decomposition of grass shoot residues in soil perturbed by copper or heat (see text for details), expressed as a percentage of decomposition in unperturbed soil, measured 1 day (i.e. the initial effect of the perturbation — resistance), 13 and 60 days (i.e. recovery after perturbation — resilience) after perturbation (n=3)[a]

Perturbation	Soil		1-day resistance	13-day resilience	60-day resilience
Copper	Grassland	One species	77 a (2.3)	79 b (3.4)	112 ab (5.3)
		Six species	84 (3.7)	81 (4.9)	92 (10.0)
	Industrial	Polluted	27 ab (4.0)	87 a* (8.6)	75 ab* (8.5)
		Uncontaminated	23 a (1.3)	28* (4.3)	34 a* (2.4)
	Agricultural	Intensive	43* (4.2)	39* (4.0)	43* (2.5)
		Organic	80* (8.2)	87* (2.0)	90* (3.0)
Heat	Grassland	One species	90 a (6.3)	100 (8.6)	121 a (9.8)
		Six species	92 (3.0)	97 (2.6)	137 (2.5)
	Industrial	Polluted	15 ab* (0.8)	46 a* (3.8)	32 b* (7.4)
		Uncontaminated	42 ab* (2.5)	74 a* (4.0)	80 b* (3.4)
	Agricultural	Intensive	75 ab* (1.6)	94 a (2.6)	92 b (1.7)
		Organic	93* (5.8)	101 (3.1)	98 (4.3)

[a] Means with the same letter are significantly different ($p<0.05$) within the same soil over time.
* Means are significantly different ($p<0.05$) between soils on the same day.

change in microbial community structure. A definitive answer could only be obtained by using other techniques, such as phospholipid fatty acid analysis or molecular approaches, in conjunction with CLPP (e.g. Griffiths et al., 1999). Bossio and Scow (1998) concluded that changes in CLPP patterns should not be equated with important changes in microbial community structure, as it may only measure the fastest growing portion of the community. The fact that the grassland soils were distinguishable by CLPP during the growing season (Dhillion and Roy, unpublished data) may reflect the importance of the carbon input. It is likely that during the growing season, readily available carbon exudation from the roots would have led to differences in the rhizosphere communities and thus a different CLPP pattern (Grayston et al., 1998). With the death of the annual plants the microbial community would no longer be affected by exudation and would rely on the turnover of soil organic matter and structural plant residues. The soils may have reverted to a 'background' microbial community and so no effect on CLPP pattern was observed. The lack of discrimination between the industrial soils, and indeed the greater utilisation of some substrates by the polluted soil, probably reflect the substrate utilisation results discussed below.

The two grassland soils did not differ in their patterns of substrate mineralisation kinetics. Grass shoot material caused a very rapid increase in respiratory activity and exhibited two respiration peaks. Marstorp (1996) noted similar respiration profiles following the addition of *Lolium multiflorum* residues to soil, and showed that the first peak resulted from the decomposition of water soluble compounds (mainly sugars, free amino acids and fructans) while the second was attributed to proteins and non-N-containing plant components. The timing of the peaks observed in this study was very similar to that reported by Marstorp (1996) who showed that the two peaks occurred after 10–15 and 30 h incubation at 20°C. The greatest respiratory responses were obtained from glucose in what was essentially a substrate-induced respiration (SIR) assay as used to measure microbial biomass (Anderson and Domsch, 1978). Rates obtained from sawdust and cellulose were much slower than from the more readily-available substrates, and in line with the peak respiration rates from cellulose observed after 12 days at 22°C by Hu and van Bruggen (1997).

Decomposition rates of the added substrates in the polluted industrial soil were significantly greater than in the uncontaminated soil. It would be expected for communities in soils contaminated with a particular pollutant to be able to decompose that compound more rapidly than those in uncontaminated soils. Thus, a form of 'community conditioning' occurs. Degradation of carbofuran, for example, is accelerated in previously treated soils (Suett, 1986). The interesting feature here is that pollution appears to have selected

for a community which is more able to degrade a range of substrates unrelated to the pollutant, including some which are structurally diverse and complex. Atlas et al. (1991) similarly observed that although biodiversity was significantly reduced in a freshwater microbial community exposed to petroleum, the generalised abilities of the population had broadened to enable effective utilisation of substrates not directly related to hydrocarbon metabolism.

The assumption that the soils chosen would differ in biodiversity was supported by the results. The one- and six-species grassland soils, collected from the rooting zone of an annual grassland after the successful growth of a crop (Dhillion and Roy, unpublished data), both contained protozoan populations typical of mineral soil (Griffiths, 1994). The industrial soils were collected from a depth of 3 m where it was anticipated that the lack of plant-derived inputs would have led to an impoverished microbial community. This was indicated by the much reduced protozoan population. The polluted soil would, intuitively, have had an even more impoverished microbial community than the uncontaminated site. This was borne out by the still lower protozoan populations, and the lack of fungal development when incubated with grass residues. Protozoa, because they feed on soil micro-organisms, are regarded as a reliable indicator of previous microbial productivity (Christensen et al., 1996) and to be a particularly sensitive indicator of microbial population change (Angle, 1994). Substrate utilisation did not reflect these differences in soil biology. The CLPP did not distinguish the uncontaminated from the polluted industrial soil, although the utilisation of individual substrates did indicate a greater metabolic potential for the polluted soil. The polluted industrial soil decomposed added substrates equally as efficiently as the grassland soils.

Functional stability, as described in the introduction, is made up of resistance and resilience, and these need to be discussed separately. The grassland soils were more resistant to both copper and heat than the industrial soils, and the uncontaminated industrial soil was more resistant to heat than the polluted industrial soil. In an earlier study of functional stability in experimentally manipulated grassland soils, there was resistance but no resilience to the copper stress (Griffiths et al., 2000). This was explained by the fact that the applied copper was always present and acted as a persistent stress. In this study there was an apparent delay in the recovery of the one-species grassland soil between 15 and 60 days after adding the copper. However, there was no indication of resilience between 1 and 15 days, and the one-species soil showed a greater resilience than the six-species soil. This may be related to the recovery of the populations following the application of the stress. The polluted industrial soil also showed resilience to copper, in that decomposition was much recovered after 15 days, and was equally so after 60 days. The fact that two of the measurements indicated recovery suggests that this is a real effect, and may reflect the extreme versatility of populations from stressed habitats (Atlas et al., 1991). There was slight resilience to heat shown by the grassland soils, and overshoot by the one-species soil in that decomposition after 60 days was higher in the heated soil than the unheated control. Such an overshoot is entirely consistent with the general ecological theory (McNaughton, 1994). The polluted industrial soil recovered to a lower value than the uncontaminated industrial soil, which in turn recovered to a lower value than the grassland soils.

The term ‘soil health’ encompasses broad general statements, for example definitions such as ‘the continued capacity to function as a vital living system’ (Doran and Safley, 1997). As more discussion is made of soil health it will become increasingly important to obtain objective and relevant measures of the concept. We argue that substrate utilisation profiles do not provide a representative measure of soil health, and indeed may not even serve as a loose indicator. The polluted industrial soil, which was compromised in terms of both biodiversity and functional stability, could utilise a broad range of substrates more effectively than grassland soil. The organically managed agricultural soil had greater functional stability than the intensively managed soil, in particular having greater resistance to the stresses than the intensively managed soil. The protozoan biomass of the intensively managed soil was equivalent to that of the grassland soils, but the grassland soils were more stable to the applied stresses than the intensively managed agricultural soil. Thus, while protozoan biomass may be able to indicate comparative soil biological status among soils of a similar type it is of limited value in comparisons between different soil types. The advantages and disadvantages of protozoa as bioindicators were discussed in detail by

Foissner (1999), who concluded that a far more detailed analysis, both in terms of taxonomy and function of the taxa would be required in order to exploit their potential as bioindicators. The same is probably true of other faunal groups. It is unlikely to be possible to define soil health with a single index, as it is a multi-faceted concept incorporating biological, physical and chemical attributes (Szabolcs, 1994). The results of this study indicate that functional stability is a useful biological parameter since it is informative of the system and interpretable in terms of soil function.

5. Conclusions

This is the first use of the functional stability assay we have devised (Griffiths et al., 2000) in field as opposed to experimental soils. The combined results of these studies suggest a number of ways in which the assay might be improved. For example, if the decomposition of the added substrates were measured on more occasions after the stresses are applied, it may be possible to parameterise the resilience time-course. In addition, the use of a wider range of stresses might reveal different aspects of the soil microbial community, and the use of different substrates could focus the assay for specific purposes. Overall, the results suggest that the assay of functional stability can potentially quantify differences in biological status between soils, and may provide the basis for a quantitative measure of soil health.

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