Soil Biology & Biochemistry 40 (2008) 2182–2185

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Short communication

Structural diversity and enzyme activity of volcanic soils at different stages of development and response to experimental disturbance

L. Shillam [a,*], D.W. Hopkins [a,b], L. Badalucco [c], V.A. Laudicina [c]

[a] School of Biological and Environmental Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK
[b] Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK
[c] Dipartimento ITAF, Università di Palermo, Viale delle Scienze 13, 90127 Palermo, Italy

ARTICLE INFO

Article history:
Received 22 October 2007
Received in revised form 31 March 2008
Accepted 7 April 2008
Available online 12 May 2008

Keywords:
PLFA
Enzyme activity
Volcanic
Development
Disturbance

ABSTRACT

We investigated the phospholipid fatty acid (PLFA) diversity and enzyme activities in soils from the volcano, Mt. Etna (Sicily). The soils were at sites which have been developing for different periods of time and have formed in volcanic lava of differing ages that have been supplemented with volcanic ejecta from subsequent eruptions. However, the plant communities indicated a marked successional difference between the sites and we have used this as a proxy for developmental stage. We have compared the structural and functional properties of the microbial communities in soils from the two sites and tested experimentally the hypothesis that the more diverse community was more resistant and resilient to disturbance. The experimental disturbance imposed was heating (60 °C for 48 h) and the recovery of enzyme activities (β-glucosidase, acid phosphatase and arylsulfatase) and structural properties (PLFA profiles) were then followed over six months. The microbial community in the soil from the older site was more structurally diverse and had a larger total PLFA concentration before disturbance than that of the soil from the younger site. The older soil community was not more resistant and resilient following an environmental disturbance as the younger soil community was equally or more resistant and resilient for all parameters. Changes in enzyme activities following disturbance were almost entirely attributable to changes in biomass (total PLFA).

The extrusion of lava during volcanic eruptions creates virgin land surfaces which are then subject to soil formation and colonisation. We have compared the structural and functional properties of the microbial communities in soils from young and old volcanic soils. We tested the hypothesis that the community in the old site was more diverse and more resistant and resilient to disturbance. Odum (1969) suggested that species number and evenness increase with ecosystem development and that there is a link between species diversity and community resistance and resilience. Although some evidence exists to suggest this for plant or animal communities (e.g. Tilman, 1996), it is not clear whether this is the case for microbial communities. Giller et al. (1997) suggested that decreases in the diversity of soil microbial community may cause a decline in resistance and resilience.

In this study we used phospholipid fatty acid (PLFA) analysis to assess community structural diversity and the activity of three soil enzymes to assess the effects of disturbance in two volcanic soils of different ages. First, we hypothesised that older soil microbial communities would be more structurally diverse and have greater enzyme activity. Second, we hypothesised that the older soil community would be more resistant and resilient following an environmental disturbance.

Etna is Europe's most active volcano. It is located in the north east of Sicily, southern Italy (15°0′ E, 37°43.8′ N). Soils were sampled from two sites at different developmental stages on the south and east facing slopes of Etna near the towns of Nicolosi and Zafferana. The sites were classified into developmental stage from field observations and previous soil biological and chemical data (Hopkins et al., 2007). The younger site was located on Monti Rossi (MR), which is a cinder cone formed in 1669, and the vegetation is now dominated by the *N*-fixing pioneer, *Genista aetnensis* (Etnean Broom). The older site was at Salto del Cane (SD), in an area dominated by mature *Castanea sativa* (European Chestnut) woodland and was formed around 7500 years ago. The mean annual precipitation in the area is 1100–1300 mm, and the mean annual air temperature is 14.5 °C, although air temperatures can rise to 35 °C in the peak of summer and fall to 0 °C in winter (Fernandez-Sanjurjo et al., 2003; Hopkins et al., 2007). Three replicate soil samples were collected at each site, sieved to less than 2 mm to remove stones and large root fragments, and then stored in sealed polyethylene bags at 4 °C for no more than four weeks. C and N contents of soils were determined using a Carlo-Erba CHN analyser and soil pH was determined on a 1:2.5 soil to water suspension. The

* Corresponding author. Tel.: +44 1786 466540; fax: +44 1786 467 843.
E-mail address: ls25@stir.ac.uk (L. Shillam).

0038-0717/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2008.04.006

Table 1
One way ANOVA for the effects of soil age on each soil parameter

Parameter	*F*-Value
PLFA diversity	669.74***
Total PLFA	81.91**
Total β-glucosidase	99.19**
Total acid phosphatase	0.41NS
Total arylsulfatase	30.65**

F values are displayed and *, ** and *** represent significance at $P < 0.05$, 0.01 and 0.001 level, respectively. NS represents no significant effect.

soil from Monti Rossi contained 19.1 mg C g^{-1} soil and 0.85 mg N g^{-1} soil, and had pH of 6.5. The soil from Salto del Cane contained 14.1 mg C g^{-1} soil and 1.06 mg N g^{-1} soil, and had pH of 6.4.

Soil samples (15 g dry weight equivalent) were weighed into glass vials and incubated at 20 °C for 10 days for equilibration. Disturbance was imposed by heating the soils in uncovered vials in an oven at 60 °C for 48 h and then allowing them to cool after which the soils were re-adjusted to their original water content. Unheated soil was used as the control. The moisture contents of both disturbed and control samples were adjusted to 50% water holding capacity and they were incubated in the dark at 20 °C for 148 days. Vials of soil were sampled destructively after 0, 2, 16, 33, 54, 81, 115 and 148 days and analysed for β-glucosidase, arylsulfatase and acid phosphatase activities. Samples collected on days 2, 33, 81 and 148 were analysed for PLFA profiles.

All soil enzyme activities were based upon the colorimetric determination of *p*-nitrophenol (Tabatabai and Bremner, 1970). The substrates for β-glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2) and arylsulfatase (EC 3.1.6.1) were *p*-nitrophenyl β-D-glucopyranoside, *p*-nitrophenyl phosphate and *p*-nitrophenyl sulphate, respectively. The methods for each of the enzyme assays are outlined in Alef and Nannipieri (1995).

Lipids were extracted from soil based on a modified single phase technique and separated into PLFAs, glycolipids and neutral lipids using silicic acid columns (Zelles, 1999). After a final clean-up, samples were analysed using a Pye Unicam PU4400 gas chromatograph (GC) with a flame ionisation detector. The detector and injector of the GC were set to a temperature of 320 °C and the column was programmed to heat in three stages from 60 °C to a maximum of 310 °C. The eluted peaks were recorded and identified with reference to a qualitative standard of bacterial acid methyl esters.

PLFA diversity was summarised using Shannon's diversity index:

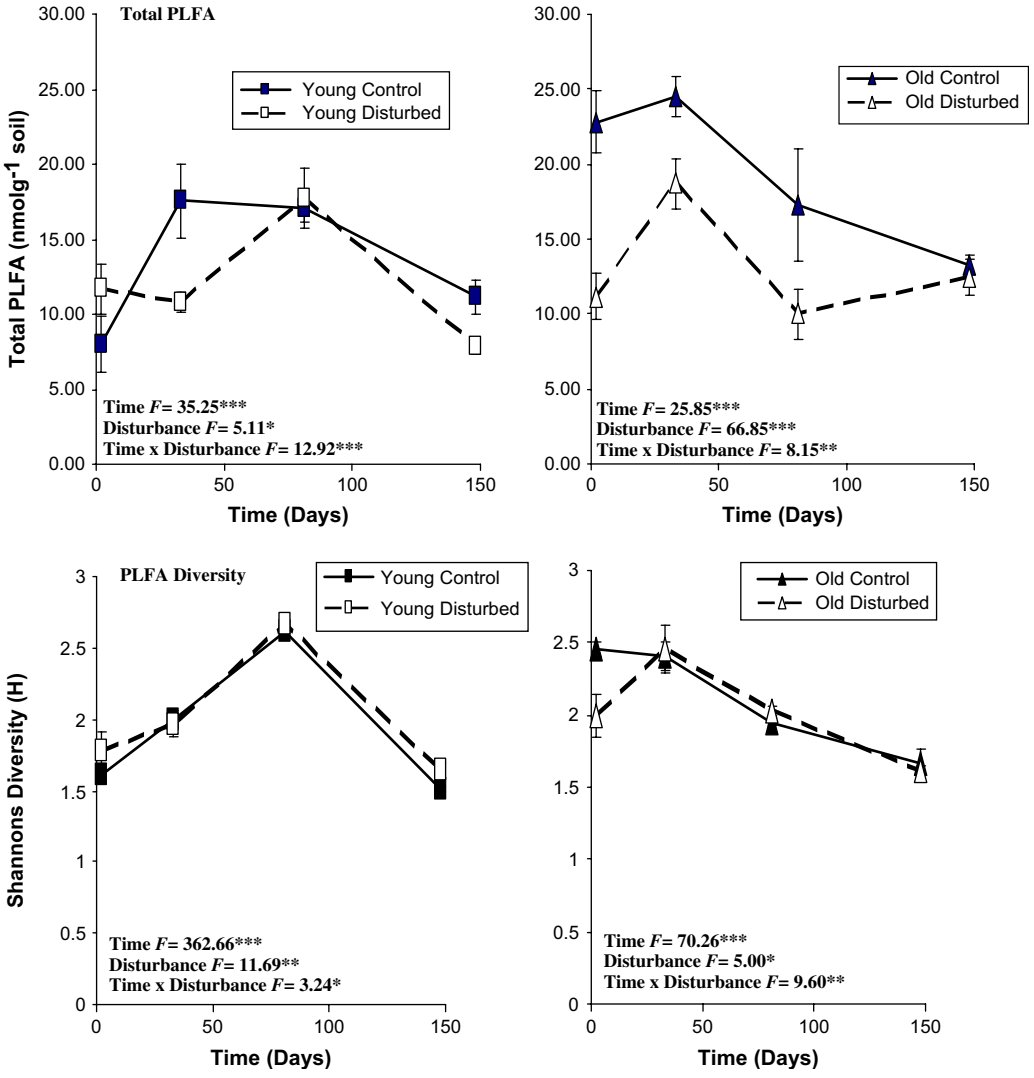

Fig. 1. PLFA diversity and total PLFA of the younger Monti Rossi soil and older Salto del Cane soil both control and after a disturbance. Values are means (±SD). Repeated measures' ANOVA for the effects of time, disturbance and time × disturbance. *F* values are displayed and *, ** and *** represent significance at $P < 0.05$, 0.01 and 0.001 level, respectively.

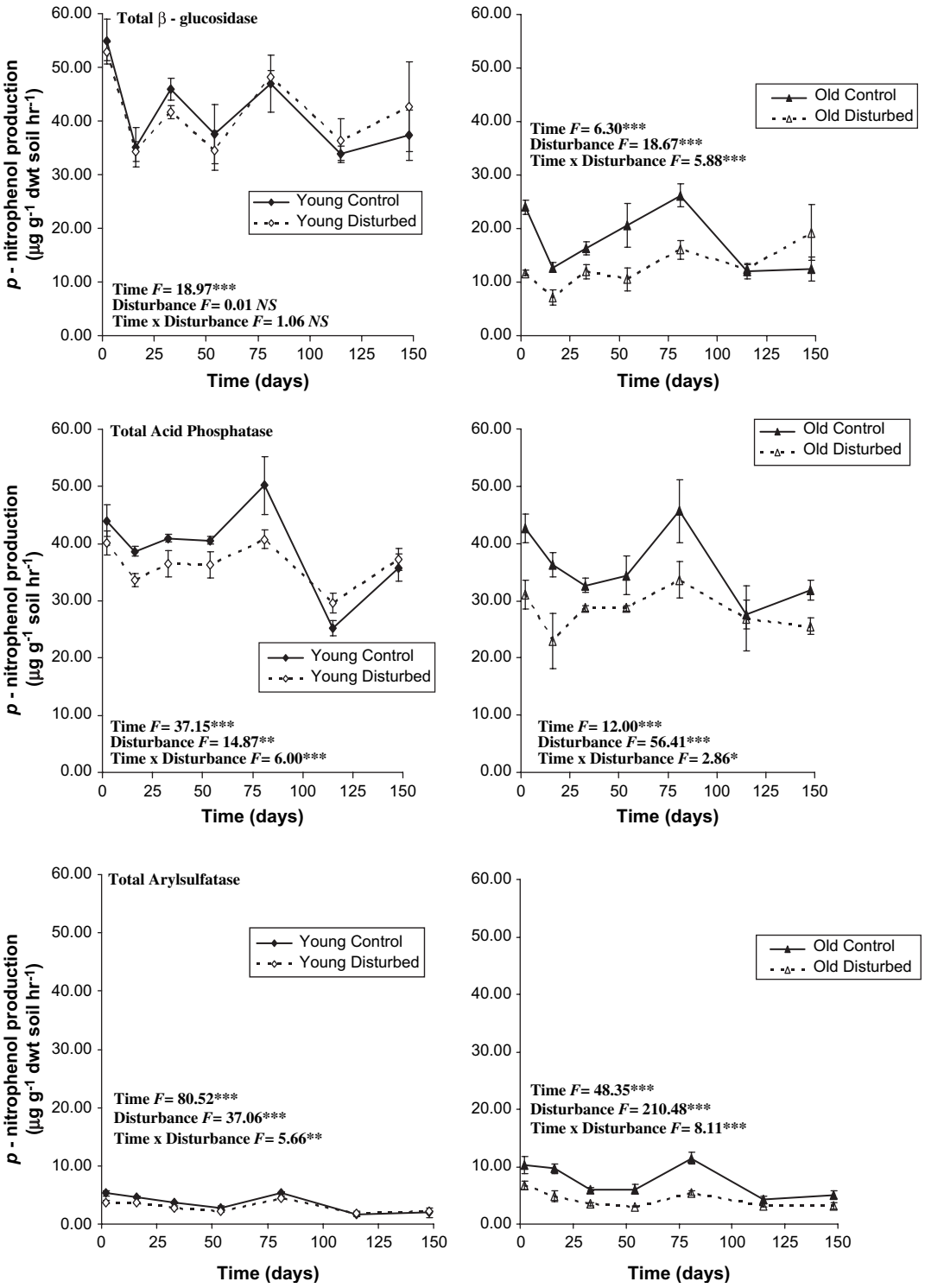

Fig. 2. Total enzyme activities of younger Monti Rossi control and disturbed soils and, older Salto del Cane control and disturbed soils. Repeated measures' ANOVA for the effects of time, disturbance and time × disturbance effects at each time period. *F* values are displayed and *, ** and *** represent significance at $P < 0.05$, 0.01 and 0.001 level, respectively. NS represents no significant effect.

$$\boldsymbol{H} = -\sum_{i=1}^{n} p_i \ln p_i$$

where n represents the number of PLFAs and p_i is the abundance of the each PLFA in the total sum.

Total PLFA concentration in the soils was used as a proxy for microbial biomass (Bååth et al., 1992).

One way ANOVA was used to assess the effect of soil age on PLFA diversity, total PLFA and enzyme activities. Repeated measures' ANOVA was used to assess the effects of disturbance and time on PLFA diversity, total PLFA and enzyme activities. All calculations were carried out on an oven dry weight basis.

The hypothesis that the older soil microbial community would be more structurally diverse was supported (Table 1). The initial PLFA profile of the older soil was significantly more diverse and

contained significantly more PLFAs than the younger soil (Fig. 1). Greater PLFA diversity in the older soil concurs with Odum's (1969) theory that species number increases during community development, and with other studies of ecosystem development (Ohtonen et al., 1999; Tscherko et al., 2004).

The hypothesis that older soils would have greater enzyme activities was not supported (Table 1). β-Glucosidase activities in the younger soil were significantly greater than in the older soil throughout the experiment (Fig. 2). By contrast, soil development had a less significant effect on acid phosphatase activity, as concentrations were similar in both soils (Fig. 2). Arylsulfatase activity was significantly greater in the older soil than in the younger soil (Fig. 2). Enzyme activities increased during the early phases of soil development on a glacial foreland sequence (Tscherko et al., 2003), with activities associated with C and N transformations subsequently declining as nutrients accumulate (Tscherko et al., 2004; Allison et al., 2007), presumably as a response to reduced nutrient limitation (Allison and Vitousek, 2005).

Greater diversity and total PLFA did not result in greater enzyme concentrations in these soils indicating that other soil properties such as nutrient concentrations (Allison and Vitousek, 2005) may be responsible for regulating enzyme production. It is also important to note that soil enzyme assays conducted under optimized conditions may include enzymes not of microbial origin and also stabilised extracellular enzymes (Burns, 1978).

We hypothesised that the older soil community would be more resistant and resilient following an environmental disturbance. This hypothesis was not supported, as after disturbance all enzyme activities, PLFA diversity and total PLFA were all equally (or more) resistant and resilient in the younger soil than in the older soil. PLFA diversity and total PLFA significantly declined in the older but not in the younger soil following disturbance (Fig. 1). Disturbance had no significant effect on β-glucosidase activity in the younger soil, but led to a reduction in activity in the older soil (Fig. 2) suggesting that β-glucosidase activity in the younger soil was more resistant. Acid phosphatase activity significantly declined in both soils as a result of disturbance but the younger soil was less affected and recovered at a faster rate suggesting it was more resistant and resilient to disturbance. Arylsulfatase in the younger soil was more resistant and resilient to disturbance than the older soil. This pattern followed changes in biomass indicating that reduced biomass was responsible for enzyme activity decline.

A system's stability determines its ability to continue functioning under changing conditions (Orwin and Wardle, 2004). The consequences of greater diversity in soils are not fully understood although more diverse communities may be more stable when subject to environmental perturbations (Giller et al., 1997). In our study, the PLFA diversity, total PLFA concentration and total enzyme activities were all less resistant in the older soil compared to the less developed soil when subject to disturbance.

In conclusion, the soil microbial community in the older soil was more diverse and had a greater PLFA diversity than the younger soil. By contrast, the enzyme activities were not greater in the older soil. All parameters in the younger soil were equally or more resistant and resilient to disturbance than in the older soil. Changes in enzyme activities following disturbance were almost entirely attributable to changes in biomass (total PLFA).

Acknowledgments

We thank Antonio Ioppollo, Riccardo Scalenghe, Giuseppe Lo Papasulla and Marco Meli for field assistance and Lorna English for laboratory assistance. This study was funded by the Natural Environment Research Council.

References

Alef, K., Nannipieri, P., 1995. Methods in Applied Soil Microbiology and Biochemistry. Academic Press Limited, London.
Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient input. Soil Biology and Biochemistry 37, 937–944.
Allison, V.J., Condron, L.M., Peltzer, D.A., Richardson, S.J., Turner, B.L., 2007. Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Joseph chronosequence, New Zealand. Soil Biology and Biochemistry 39, 1770–1781.
Bååth, E., Frostegård, A., Fritze, H., 1992. Soil bacteria biomass, activity, phospholipid fatty acid pattern, and pH tolerance in an area polluted with alkaline dust deposition. Applied and Environmental Microbiology 58, 4026–4031.
Burns, R.G., 1978. Soil Enzymes. Academic Press, London.
Fernandez-Sanjurjo, M.J., Corti, G., Certini, G., Ugolini, F.C., 2003. Pedogenesis induced by *Genista aetnensis* (Biv.) DC. on basaltic pyroclastic deposits at different altitudes, Mt. Etna, Italy. Geoderma 115, 223–243.
Giller, K.E., Beare, M.H., Lavelle, P., Izac, A.N., Swift, M.J., 1997. Agricultural intensification, soil biodiversity and agroecosystem function. Applied Soil Ecology 6, 3–16.
Hopkins, D.W., Badalucco, L., English, L.C., Meli, S.M., Chudek, J.A., Ioppolo, A., 2007. Plant litter decomposition and microbial characteristics in volcanic soils (Mt Etna, Sicily) at different stages of development. Biology and Fertility of Soils 43, 461–469.
Odum, E.P., 1969. The strategy of ecosystem development. Science 164, 262–270.
Ohtonen, R., Fritze, H., Pennanen, T., Jumpponen, A., Trappe, J., 1999. Ecosystem properties and microbial community changes in primary succession on a glacier forefront. Oecologia 119, 239–246.
Orwin, K.A., Wardle, D.A., 2004. New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. Soil Biology and Biochemistry 36, 1907–1912.
Tabatabai, M.A., Bremner, J.M., 1970. Factors affecting soil arylsulfatase activity. Soil Science Society of America Proceedings 34, 225–229.
Tilman, D., 1996. Biodiversity: population versus ecosystem stability. Ecology 77, 350–363.
Tscherko, D., Rustemeier, A., Richter, A., Wanek, W., Kandeler, E., 2003. Functional diversity of the soil microflora in primary succession across two glacier forelands in the Central Alps. European Journal of Soil Science 54, 685–696.
Tscherko, D., Hammesfahr, U., Marx, M., Kandeler, E., 2004. Shifts in rhizosphere microbial communities and enzyme activity of *Poa alpina* across an alpine chronosequence. Soil Biology and Biochemistry 36, 1685–1698.
Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils 29, 111–129.