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Abundance and diversity of soil arthropods and fungi in shelterbelts integrated with pastures in the central tablelands of New South Wales, Australia

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ABSTRACT: Shelterbelts are important for the sustainability of agriculture because they provide a variety of benefits to farmers and the society. Several published papers demonstrate that integration of shelterbelts with agroecosystems offers positive outcomes, such as better yield, more congenial microclimate, and greater organic matter levels. Nonetheless, soil biological diversity, the driver of greater organic matter levels, has not been convincingly tested and verified yet. In addressing this gap, we measured abundance and diversity of populations of arthropods and fungi in three 11-year old shelterbelts integrated with pasture to determine whether a correlation exists between the abundance of and diversity in populations of arthropods and fungi in two seasons: late autumn-early winter (May–June 2011) and late winter-early spring (August–September 2011). Litter from the soil surface and soil from two depths were sampled at increasing distance from the midpoint of shelterbelts for the extraction of arthropods and isolation culturing of fungi. The relationship among distance, depth and biodiversity of different groups of arthropods and fungi was analysed using linear regression. We found that over both seasons arthropod abundance in the litter and soil declined with increasing distance from the midpoint of the shelterbelts, and with soil depth. However, fungi abundance in either season was not affected by proximity to the shelterbelt but increased with greater soil depth. Distance from the shelterbelt midpoints did not bear an impact on the diversity richness of both arthropods and fungi.

Keywords: diversity richness; shelterbelts; pastures; soil arthropods; soil fungi; species abundance

Agroforestry provides ecosystem services, environmental benefits, and economic commodities as part of a multifunctional landscape (Jose 2009). Shelterbelts, either as single plantings or as part of an agroforestry system, provide a sustainable link between agricultural production and natural-system conservation (Mize et al. 2008), besides providing several other benefits (Bennel, Verbyla 2008). For example, yield increases up to > 20% could be achieved in proximal crops in the area that stretches beyond shelterbelts by 10 times the average height of trees in shelterbelts (Cleugh 2003). Shelterbelts also improve microclimatic factors such as temperature and humidity, increase organic matter and enable trapping of sediments and water, increase biodiversity enabling better pest management, provide better wildlife habitat, and facilitate greater levels of carbon sequestration (Brandle et al. 2004).

The role of shelterbelts in enabling a measurable impact on soil biological diversity, because of enhanced habitat diversity, stands as a supposition (Walcott 2004). This supposition is founded on the understanding that the more diverse the soil biota, the better are soil-turnover rates, mineralization and humification of organic matter, soil texture and consistency, infiltration rate, and soil-water retention characteristics (Bardgett, Wardle 2010). Among different soil-biotic elements, arthropods and fungi exhibit greater levels of abundance and diversity, because they occupy a more extensive range of microhabitats and niches than other soil

organisms (Fitter 2005). For example, Eisenbeis (2006) showed more than 1,000 invertebrate species in temperate woodland soils, including an extensive range of arthropods, with an overall abundance of millions per m^2 in the top 5 cm. The high diversity of soil and litter arthropods is not only because of the heterogeneous characteristics of soil and litter, but also because of particle size that enables different species to occupy different niches within them (Sarty et al. 2006). Fungi, on the other hand, are also equally vital components of soil ecology; they occur in nearly all situations, especially in sustainably managed soils (Bridge, Spooner 2001). The mycorrhizal fungi in crop lands, for example, show a variety of beneficial effects on the crop plants by enhancing their mineral-uptake capacity (Jeffries et al. 2003).

Either a subtle or an obvious change in an ecosystem influences the distribution, diversity and abundance of arthropods and fungi, because they are highly sensitive to changes facilitated by biotic and abiotic factors (e.g. Mulder et al. 2005). Shelterbelts, in principle, are a modification introduced into an agroecosystem to improve productivity and longer-term stability (Méndez 2001). Local habitat conditions (e.g. microclimate, physical and chemical traits of soil) such as the aboveground floristic diversity and structure (De Bellis et al. 2007; Palacios-Vargas et al. 2007; Sylvain, Wall 2011), temperature (King et al. 1998), humidity (Chikoski et al. 2006), soil chemistry and structure (Boulton et al. 2005), and quantity and composition of litter (Treseder 2005) drive the organization of arthropod and fungal communities more strongly than large-scale landscape traits (Dauber et al. 2005). Moreover, arthropod and fungal populations mutually influence each other: herbivorous arthropods selectively influence soil fungal communities (Crowther et al. 2011). These arguments reinforce that soil biotic elements, especially the arthropods and fungi, serve as useful indicators of soil structure and health (Doran, Zeiss 2000; Bardgett et al. 2005, also see Commission on Genetic Resources for Food and Agriculture 2002 for an extensive list of citations).

Effect of distance from the shelterbelt into an agroecosystem has always been of interest (Kohli et al. 1990; Ekpe, Bashir 2005). The effect of distance from the shelterbelt into neighbouring agroecosystems in Australia has been demonstrated in the context of abundance of natural enemies of arthropods that regulate populations of pestiferous arthropods (Gámez-Virués et al. 2009; Thomson, Hoffman 2009). The Dorrough et al. study (2004) made in Australian temperate grassy landscapes integrated with shelterbelts emphasized improved production (ca 30%) up to a distance of 12 times the average height of the shelterbelt tree taxa.

Nevertheless, few studies have attempted to measure the effects of shelterbelts on the biological diversity of soil arthropods and fungi. Against such a background, our study seeks to explore whether any correlation exists between the abundance of and diversity in the arthropod fauna and fungi in the litter and soil of pasture and distance from the associated shelterbelt. We were convinced that such a link would be a useful new tool in evaluating the impacts and benefits of shelterbelts in an agroecosystem (sensu Paoletti 1999).

MATERIAL AND METHODS

Study site and experimental design

The study was done at the Orange campus farm of Charles Sturt University (33°15'S; 149°07'E; 875 m a.s.l.) in the central tablelands of New South Wales. Soil types range from red-brown silty clay loam Dermosols along the mid-lower slopes to Red Chromosols along the upper slopes and ridges (Kovač, Lawrie 1990; Isbell 1996). The climate is characterized by cold wet winters (2–10°C) and mild summers (12–25°C). Usually, rainfall occurs uniformly through the year (700–950 mm) (Bureau of Meteorology 2011). Mean monthly maximum temperatures in 2011 ranged from 15.1 to 18.4°C indicating that temperatures were warmer than the comparable 30-year average of 15.6°C; average rainfall was 603.4 mm in 2011, nearly 90 mm more than the preceding 30-year mean rainfall (Bureau of Meteorology 2011). Rainfall and temperature data were obtained from the weather station of the University campus.

Three shelterbelts (Leeds Parade, College 4, and Weston 1; hereafter referred as Sites 1, 2, and 3, respectively), each about 100 m long and 15–25 m wide, established in 1998–2000 and situated within previously established pasture land, were used. The mean above-sea elevations are 885 m a.s.l. for Sites 1 and 2, and 907 m for Site 3. These sites were chosen for their similarity in being narrow, elongated shelterbelts with a northerly-southerly orientation, receiving the same easterly downwind, in which the sampling transects were constructed. The principal purpose of establishing these shelterbelts with a mixture of seedlings of Australian native trees was to capitalize on the advantages the shelterbelts provide when occurring in a pasture ecosystem

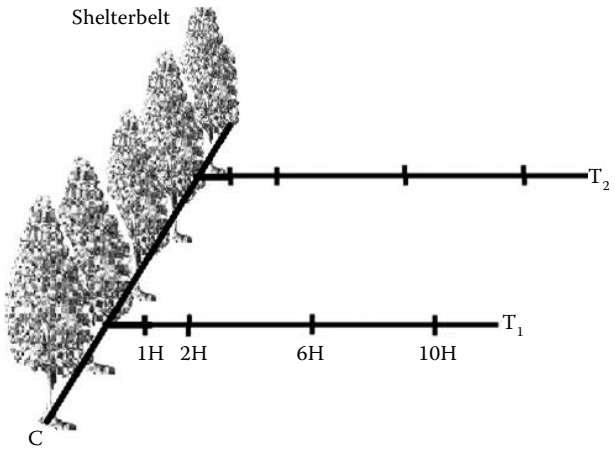

Fig. 1. Representative diagram depicting details of the two transects T_1, T_2, sampling points used at each site (not to scale)
C – midpoint of the shelterbelt, T – transect, H – tree height (1H – one tree height, 2H – two tree heights, 6H – six tree heights, 10H – 10 tree heights)

of perennial pasture taxa. Populations of *Phalaris aquatica*, *Lolium rigidum*, *L. perenne*, *Holcus lanatus* (all Poaceae), and *Trifolium subterraneum* (Fabaceae) are the principal species in pasture. Populations of *Trifolium repens* (Fabaceae), *Vulpia bromoides*, and *Hordeum glaucum* (Poaceae), and *Echium plantagineum* (Boraginaceae) occur in fewer numbers than the previously listed taxa of Poaceae and Fabaceae. Shelterbelt taxa include *Eucalyptus blakelyi*, *E. macrorrhyncha*, *E. melliodora*, *E. pauciflora*, *E. viminalis* (Myrtaceae), *Acacia dealbata*, *A. implexa*, *A. vestita* (Mimosaceae), *Casuarina cunninghamiana* (Casuarinaceae) and large-native shrubs *Callistemon sieberi* and *Leptospermum myrtifolium* (Myrtaceae). Due to minor differences at the times of planting, the mean tree heights of plants at Sites 1, 2, and 3 varied from 4.6 to 6.4 m. Therefore, average tree heights at each site were used as a pertinent measure to determine the distances for various sampling points to extract soil arthropod and fungal populations. Tree heights were determined by obtaining average clinometric readings for randomly selected 15 trees, following the procedure described by the Department of Environment and Climate Change (n.d.).

Two parallel-running transects (T_1, T_2) situated at 90° to the midline of the shelterbelt, separated by a distance of 30 m, were constructed at Sites 1, 2, and 3. Five sampling points, named Zero, 1H, 2H, 6H and 10H, starting from the midline of each shelterbelt were used (Fig. 1). The mean tree height at Site 1 was 4.6 m; that at Site 2 was 6.4 m, and that at Site 3 was 5.6 m; the sampling points – the distance variables – 1H, 2H, 6H and 10H, were calculated based on mean tree heights (Table 1). The midline sampling point is shown as the zero point on the x axis of Fig. 2. Thus, two of the five sampling points (zero and 1H) fell within the shelterbelt vegetated area; the remaining sampling points fell within the adjacent pasture. A maximum of the equivalent of 10 tree heights was chosen as the most-distant sampling point, following Cleugh (2003). A similar distance factor has been reinforced for aboveground abundance and diversity measurement by Crowther et al. (2011).

Sampling

Sampling of arthropods and fungi was done twice synchronizing with seasons in central-west NSW in 2011 as shown: on 26 May (Site 1), 3 June (Site 2), and 14 June (Site 3) to synchronize with late autumn-early winter; on 15 August (Site 1), 29 August (Site 2), and 9 September (Site 3) to synchronize with late winter-early spring.

Arthropods

Arthropods from litter (0–1 cm) and from two soil depths (1–10, 11–20 cm) were obtained from each of the sampling points: Zero (centre of shelterbelt), 1H, 2H, 6H, and 10H. Litter material was sampled from 25 × 25 cm quadrats using a vacuum sampler (Weed Eater®, Model GB1 30v, Poulan Co., Shreveport, USA; collecting duct diameter size: 10 cm). Samples were collected running the motor of the vacuum sampler at its maximal speed for 60 sec (Tom et al. 2006). Soil samples from 1–10 cm and 11–20 cm depths were collected digging the soil with a 10-cm wide auger. Arthropods from the litter samples and from 1–10 cm and 11–20 cm depths were separated using Berlese-Tullgren extractors. The litter samples were placed in the mouth of the funnel (22 cm dia) on a copper sieve (2 mm mesh), which was placed at the

Table 1. Details of the distance variable measured from the midpoint of Sites 1, 2, 3

Site	Mean tree height	1H	2H	6H	10H
	(m)				
1	4.6	4.6	9.2	27.6	46
2	6.4	6.4	12.8	40.8	64
3	5.6	5.6	11.2	33.6	56

1H – one tree height, 2H – two tree heights, 6H – six tree heights, 10H – 10 tree heights

junction between the tapering end of the mouth and the stem of the funnel. Before the soil samples were placed in the Berlese-Tullgren extractors, clods of soil were manually broken to enable an easy movement of arthropods. The funnels of the extractors holding either soil or litter materials were covered with metal lamp shades fitted with 25W burning incandescent lamps. The extractors were left undisturbed for 10 days. The arthropods collected in 70% alcohol-including 100 ml conical flasks placed at the bottom of the stems of each extractor were identified to their respective orders using Harvey et al. (1989); numbers were counted and logged.

Fungi

For measuring fungal populations, samples from different depths (0–1, 1–10, and 11–20 cm) were obtained from each sampling points. The collected samples were cultured in sterile Petri plates containing potato-dextrose agar (PDA) using Warcup's soil-plate method (Bronicka et al. 2007). PDA was prepared by suspending 39 g of PDA extract in 1 l distilled water. PDA extract was dissolved by heating and stirring using a magnetic stirrer for 30 min. The medium was then sterilized in an autoclave at 121°C and 1 kg·cm^{-2} pressure for 20 min. After autoclaving, 30 mg of streptomycin (Streptomycin sulphate, S9137, Sigma-Aldrich, Castle Hill, NSW) was added to restrict bacterial growth (Bronicka et al. 2007). Soil plates were prepared by transferring 0.01 g of either soil or litter (as appropriate) into sterile Petri plates and stored in laminar airflow (Clyde-Apac, Model RFC 90, Crown Scientific, Minto, NSW). Three Petri plates were prepared for inoculating samples from each sampling site. Ten ml of air-cooled PDA was added using a sterile syringe. Each Petri plate was gently gyrated in the laminar airflow to disperse the soil (or litter) materials evenly on the culture medium. The plates were incubated at 25°C for five days and colonies growing in the agar were identified under a dissecting microscope to the genus following Watanabe (2002). The taxa that could not be determined with Watanabe (2002) were classed as RTU 1, 2, 3, and so on (RTU: recognizable taxonomic unit, sensu Oliver, Beattie 1993).

Statistical analysis

Margalef diversity index D_{Mg} was used to measure diversity richness, using the formula:

$$D_{Mg} = \frac{(S-1)}{\ln N}$$

where:

S – number of either arthropod orders or fungal genera which were present,

N – total number of individuals in the obtained sample,

ln – natural logarithm.

Because the Margalef diversity index has no limit value, it shows variations depending on the numbers of either arthropod orders or fungal genera. Hence, it was applied by comparing diversity patterns (Margurran 2004) at the three investigated sites.

Statistical tests were done using GenStat® for Windows (Payne et al. 2003). Numbers of arthropods and fungi were square-root transformed to meet the normality assumption. A multiple-regression model considering the variables distance, site, depth, interaction between distance and depth, distance and site, depth and site, and distance and depth and site was fitted for total arthropods and fungi. Stepwise regression showed that distance, depth, and distance × depth were the only significant factors ($P < 0.05$) for both arthropods and fungi in both seasons. Hence the site, distance × site, depth × site, and distance × depth × site factors were not considered in further analyses. Square root (total number) and the Margalef diversity index of arthropods and fungi were used as dependent variables.

RESULTS

Arthropod abundance (late autumn–early winter 2011)

A total of 13,999 individual arthropods were extracted. Arthropods belonging to the orders Protura and Acarina constituted 64% (total number 8959) and 20% (2800) of the extracted arthropods, repsectively. The remainder (counted number of individuals shown in brackets) were determined as belonging to the Thysanoptera (558), Coleoptera (436), Hymenoptera (292), Diplura (292), Collembolla (201), Dermaptera (122), Araneae (76), Lepidoptera (24), Diptera (24), Isopoda (24), Hemiptera (27), Chilopoda (18), Orthoptera (8), Isoptera (6), Diplopoda (3), Psocoptera (3), and Dictyoptera (1), and unidentified taxa (8). Linear regression relating abundance (square root of total) accounted for 45.6% of the variance. A significant decrease in the abundance of arthropods occurred with increasing distance from the shelterbelt and increasing soil depths (Fig. 2a).

Arthropod diversity (late autumn–early winter 2011)

Linear regression analysis of the Margalef-diversity index showed that diversity richness was not influenced by distance from the shelterbelt, whereas diversity richness decreased ($P < 0.001$) with increasing depths. The mean diversity was 1.41 for the litter, 1.20 for 1–10 cm and 0.74 for 11–20 cm.

Arthropod diversity (late winter–early spring 2011)

A total of 19 arthropod orders were identified in Sites 1, 2, and 3. Linear regression analysis of the Margalef-diversity index showed that diversity richness was not influenced by distance from the shelterbelt, whereas diversity richness decreased ($P < 0.001$) with increasing depths. The mean diversity was 1.15 for 0–1 cm, 0.99 for 1–10 cm and 0.62 for 11–20 cm.

Arthropod abundance (late winter–early spring 2011)

A total of 9,414 individual arthropods belonging to 19 orders were extracted and identified from the three sites. The most abundant orders in the samples were Protura 66% (6,255), Acarina 16% (1,541), Diplura 4.8% (439), Collembola 3.2% (300), Coleoptera 4% (364). The remainder constituted 6% of the total number of individual arthropods, belonging to the Thysanoptera (248), Hymenoptera (68), Chilopoda (16), Araneae (13), Diptera (11), Dictyoptera (6), Hemiptera (5), Lepidoptera (4), Diplopoda (2), Dermaptera (1), Heteroptera (1) and Isopoda (1). Linear regression analysis considering distance, depth and distance × depth interaction explained 55.2% of variance. No significant interaction occurred in the variable distance × depth, but both distance and depth showed significant decreases in abundance (Fig. 2b).

Fungal abundance (late autumn–early winter 2011)

A total of 9,155 colonies belonging to 10 species of fungi were isolated and identified from the three sites. An undetermined species of *Trichoderma* (Ascomycota: Hypocreales: Hypocreaceae) was the most abundant, constituting 84% (7,717), followed by a species of *Scopulariopsis* (Ascomycota: Microascales: Microascaceae) (549), a species of *Fusarium* (Ascomycota: Hypocreales: Nectriaceae) (499), a species of *Mucor* (Zygomycota: Mucorales: Mucoraceae) (387), and a few other taxa (53). The least abundant species classed under others included a species of *Humicola* (Ascomycota: *Incertae Sedis*), one of *Penicillium* (Ascomycota: Eurotiales: Trichocomaceae), *Aspergillus* (Ascomycota: Eurotiales: Trichocomaceae), *Mortierella* (Zygomycota: Mortierellales: Mortierellaceae), *Melanospora* (Ascomycota: Melanosporales: Ceratostomataceae), and one of *Chetomium* (Ascomycota:

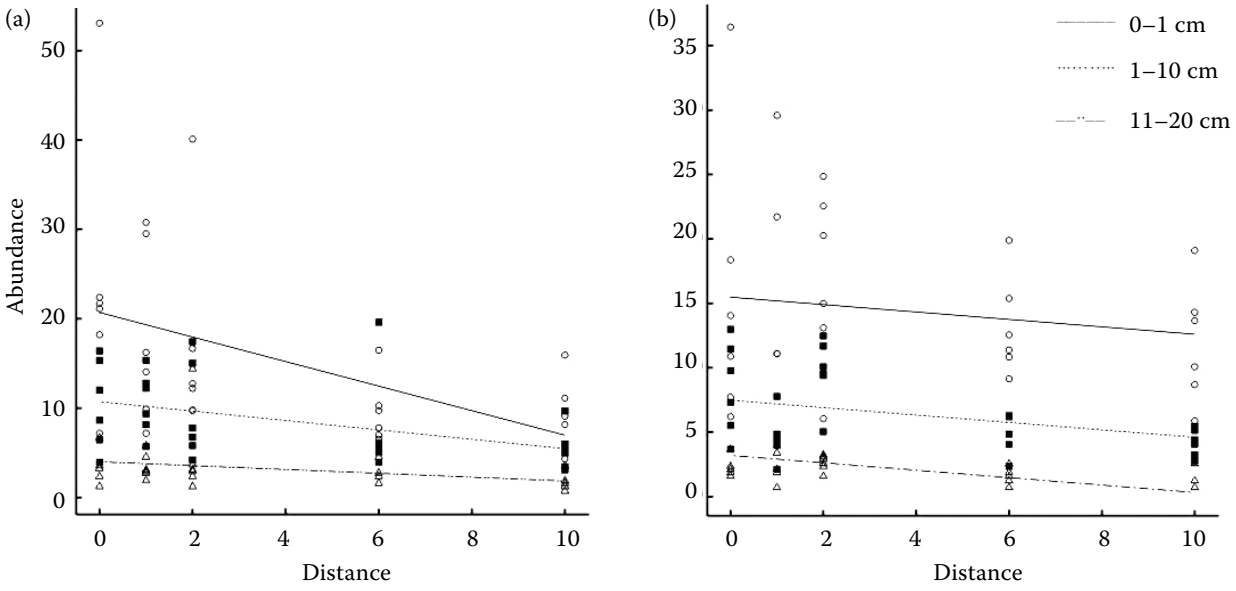

Fig. 2. Fitted and observed relationships for arthropod abundance (square root of total) in the late autumn-early winter season in relation to distance and depth factor in late autumn-early winter (a) and in the late winter-early spring season in relation to distance and depth factor in late winter-early spring (b)

Sordariales: Chetomiaceae). Linear regression analysis considering the distance, depth, and distance × depth interaction showed that distance had no effect ($P > 0.05$) on fungal abundance; however, abundance increased ($P < 0.05$) with increasing depth. The mean diversity was 6.0 for 0–1 cm, 10.3 for 1–10 cm and 11.8 for 11–20 cm.

Fungal diversity (late autumn–early winter 2011)

A total of 10 fungal taxa were isolated from pure cultures from the three study sites. Linear regression analysis of the number of genera applying the Margalef-diversity index against distance from the shelterbelt showed no significant effect of distance and depth ($P > 0.05$) on the diversity of fungi.

Fungal abundance (late winter–early spring 2011)

A total of 7,007 fungal colonies were identified from the three sampling sites with a species of *Trichoderma* with the highest number of colonies 87% (5,788), followed by a species of *Fusarium* (386), species of *Mucor* (331), species of *Scopulariopsis* (309), and a few other taxa (193). The least abundant group included species of *Humicola*, *Penicillium*, *Aspergillus*, *Melanospora*, *Chetomium*, RTU-s 1 and 2. Linear regression analysis considering the distance, depth, and distance × depth interaction showed that distance had no significant effect ($P > 0.05$) on fungal abundance, but abundance increased with increasing depth ($P < 0.05$) . The mean diversity was 5.6 for 0–1 cm, 8.8 for 1–10 cm, and 9.2 for 11–20 cm.

Fungal diversity (late winter–early spring 2011)

A total of 11 fungal genera were isolated from pure cultures from the three study sites. Linear regression analysis of the number of genera (Margalef-diversity index) against distance from the shelterbelt showed no significant effect of distance and depth ($P > 0.05$) on the diversity of fungi.

DISCUSSION

This study gains in relevance because few studies have measured the effects of shelterbelts on the abundance and diversity of soil arthropods and fungi in agricultural ecosystems. This study sought to establish correlations that existed in terms of abundance of and diversity in the arthropod fauna and fungi in the litter and two different soil depths in pasture and distance from the associated shelterbelt.

In general, the late winter-early spring samplings offered less significant results compared with those of the late autumn-early winter. We expected the diversity and richness of soil arthropods and fungi to be the greatest in the shelterbelts, and their diversity and richness to decline with increasing distance into the pasture. This expectation was proved true only for arthropods in the late autumn-early winter sampling, and in the late winter-early spring sampling. The expectation did not apply to either fungal richness or fungal diversity. Soil depth emerged as a critical element in the context of arthropod abundance: numbers of taxa belonging to Acarina, Diplura, Protura, Collembola, Araneae, Hemiptera, Coleoptera, and Lepidoptera declined ($P < 0.05$) with increasing soil depths. Late autumn-early winter for sampling arthropods showed greater richness with (*i*) increasing distance from the shelterbelt into the pasture and (*ii*) upper soil levels (0–1 and 1–10 cm depths) only. Abundance of fungi in the late autumn-early winter sampling showed no significant variation with increasing distance from shelterbelts. However, fungal abundance increased with greater soil depths. In both sampling periods, distance from the shelterbelt had no effect on fungal diversity richness.

Microarthropods such as the Collembola, Protura usually occur plentifully in soils of natural forest ecosystems (Chown, Nicolson 2004). Modified systems such as agroecosystems support either similar or lower densities of Collembola than the natural ecosystems, when they occur on soils that would be identical to the nearby natural forest ecosystems (Olejniczak 2004). High intensity of management involving extensive application of pesticides, herbicides, and mineral fertilizers affects microarthropod abundance and richness in agricultural soils (Rusek 1998). On the contrary, in organically managed agricultural fields microarthropod populations, their density and biomass, have always been consistently and significantly higher (Reddersen 1997). Our results of decreasing density of microarthropods from the shelterbelt midpoint to the middle of pasture match with the results of Reddersen (1997) and Olejniczak (2004). Livestock treading (Seniczak et al. 2007) and sporadic chemical applications for plant pro-

tection (Gergócs, Hufnagel 2009) could be the other factors that have influenced the decline in populations of microarthropods in pasture, compared with those in shelterbelts. It is also worthy of note that the increase in arthropod abundance within and closer to the shelterbelts, which occurred in the early phase of the shelterbelt – pasture (i.e. within 10 years of establishment), points favourably to the potential use of arthropods as an indicator of changes to soil health under modified agroecological systems.

In an eight-year study (1998–2006) done in Western Poland, fungal species composition changed significantly in the agroecosystem, although the tree composition in the tested shelterbelts was similar (Kujawa, Kujawa 2008); they have concluded that the age of the shelterbelts determined variations in the composition of fungal taxa. A similar conclusion is available in Lu et al. (1999) study measuring species richness of ectomycorrhizal fungi in differently aged shelterbelt stands of *Eucalyptus globulus* in Western Australia. In the present study, tree composition and age of trees were nearly identical, although tree heights varied modestly mainly due to specific geomorphic features of the landscapes in which they occur. Given that the tree age is a critical factor in determining patterns of diversity in fungal species composition, the lack of diversity in fungal species composition in the shelterbelts in Orange (NSW) does not surprise.

Increasing land-use intensification has been demonstrated to induce changes in the functional diversity of soil biota (e.g. decomposer larvae of the Syrphidae) represented as declining functional group richness, although species richness could remain stationary (Schweiger et al. 2007; also see Philpott et al. 2008). Similar trends have been shown in functional and species diversity of fungi as well (Klironomos et al. 2000). Abundance and diversity of arthropods, in particular, and fungi as useful soil-health indicators enable us to monitor the quality and sustainable function of agroecosystems and to evaluate different remediation processes that may need to be placed to achieve better performance over time.

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