Soil Biology & Biochemistry 57 (2013) 713–719

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

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

Evenness and plant species identity affect earthworm diversity and community structure in grassland soils

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ARTICLE INFO

Article history:
Received 3 April 2012
Received in revised form
24 June 2012
Accepted 25 June 2012
Available online 20 July 2012

Keywords:
Biodiversity
Evenness
Soil
Aboveground–belowground interaction

ABSTRACT

Diversity of plant species and the species composition (identity) are known to influence below-ground diversity. In this paper we examine the effects of plant species diversity (richness and evenness), rates of nitrogen application and planting density, on earthworm community structure in grassland. The study was carried out at three sites in Ireland using a Simplex experimental design to define the compositions of the experimental plant communities used.

A negative relationship was detected between diversity (evenness) of plant species and diversity of earthworms in the soils. However, plant species identity also affected the structure of the earthworm assemblage. In particular, the legume, *Trifolium repens* had a strong effect but this was conditional on the rate of nitrogen application. No earthworm species favoured communities dominated by slow growing grasses (*Phleum pratense* and *Dactylis glomerata*) ($P = 0.02$).

Higher N inputs reduced earthworm abundance and biomass under *T. repens*. Earthworm richness, was negatively influenced by elevated amounts of N inputs. No effect of planting density was detected but this factor also did not affect plant biomass production.

1. Introduction

Earthworms play many important roles in the soil processes (Edwards and Bohlen, 1996) and their digestion and defecation processes affect soil fertility (Syers et al., 1979). They are responsible for mixing organic material in the upper soil layers with intestinal secretions, including enzymes, leading to the formation of casts which influence soil structure, microbial abundance and activity and rates of decomposition and nutrient dynamics (Sheehan et al., 2008; McInerney and Bolger, 2000). In addition, channels created by earthworms, as a result of their active burrowing, improve aeration, water infiltration and reduce water runoff (Ehlers, 1975). They improve drainage (Urbánek and Doležal, 1992) and enhance soil stability (Ketterings et al., 1997).

Earthworms can stimulate the transfer of carbon and nitrogen to the above-ground parts of plants causing an increase in the plant productivity as reflected in biomass growth (Edwards and Bohlen, 1996; Derouard et al., 1997; Schmidt and Curry, 1999) and increase shoot:root ratio (Scheu, 2003).

While this positive effect of earthworms on plants has been shown in many field and laboratory studies, only a small number of studies have examined the effects of plant species on earthworms. However, recent work shows that plant community composition affects the soil fauna (Wardle, 2002, 2005; Wardle et al., 2003, 2004, 2006; Wardle and van der Putten, 2002). Thus, plants might be expected to influence earthworm populations. Because earthworms possess enzymes capable of decomposing chitin and oligosaccharides, they can contribute to the decomposition of plant litter (Parle, 1963). However, earthworms as decomposers require food with a relatively high nitrogen content, therefore they may prefer plant residues with low C:N. Grasses are often relatively nitrogen poor while legumes, because of an ability to fix nitrogen, are more nitrogen rich and recent experiments have shown that earthworm populations are enhanced by legumes (Curry et al., 2008). For example, while Schmidt and Curry (2001) found relatively small shifts in species composition between wheat and wheat-clover inter-crops, population densities increased from 319 individuals m^{-2} (55 g biomass m^{-2}) to 1160 individuals m^{-2} (175 g biomass m^{-2}). Thus, various plant species have differing effects on

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http://dx.doi.org/10.1016/j.soilbio.2012.06.016

earthworm diversity in the soil. In addition, evenness appears to be the important determinant in many such interactions (Kirwan et al., 2007; Ward et al., 2010). Therefore, in the present study we examine the effects of altering the identity and relative proportions (evenness) of grassland plant species on earthworm diversity, abundance, biomass and community structure using a novel experimental design based on a Simplex Design (Cornell, 1990). In addition, the effects of N-fertilisation and planting density, were investigated. The following hypotheses were tested: 1) plant species identity and evenness (relative abundances) will affect the structure of the earthworms assemblages, 2) the effects of variation in plant composition will be dependent on the rate of nitrogen addition which, in itself, may affect the earthworm populations, 3) the effects of variation in plant composition will be dependent on the planting density which, in itself, may affect earthworms in the soil.

2. Materials and methods

2.1. Experimental design — simplex design

A comparatively new experimental design, the simplex design (Cornell, 1990; Sheehan et al., 2006; Kirwan et al., 2007; Connolly et al., 2009), was used and proportions of all plant species were changed in addition to species richness (Cornell, 1990). The design allows the effects of species richness, evenness and species identity to be distinguished and relatively small numbers of samples are required (Sheehan et al., 2006; O'Hea et al., 2010).

The experimental design used a community set consisting of four plant monocultures Grass 1- *Lolium perenne,* Grass 2- *Phleum pratense* or *Dactylis glomerata,* Legume 1-*Trifolium pratense* or *Trifolium repens* and Legume 2- *T. repens* or *Trifolium ambiguum*, respectively (G_1, G_2, L_1, L_2) and eleven mixtures of the two grass (G_1, G_2) and two legume species (L_1, L_2). The plant species used at each of the three sites are shown in Table 1. The species were selected as being fast and slow growing legumes and grasses at the particular site (c.f. Connolly et al., 2009).

The eleven mixtures included either equal proportions of each of the four plant species (the centroid), or four mixtures dominated by each species sown in the proportion of 70% of dominant and 10% of each of the other species or six more even mixtures which consisted of 40% of each of two plant species and 10% of the other two plant species.

This design can be represented by a tetrahedron (Fig. 1) where each corner represents a monoculture of one of the four plant species, and eleven points within the tetrahedron where each point represents a community with its position being determined by the relative abundance sown (evenness E) of the four species. In this experiment different values of evenness, as defined by Kirwan et al. (2007), are used (0, 0.64 and 0.88 and 1). $E = 0$ characterises monocultures, because they contain 100% of a given species, and therefore, are highly uneven. The most even assemblages are equal mixtures (containing 0.25, 0.25, 0.25, and 0.25), thus evenness equals 1 ($E = 1$). For mixtures dominated by one species, for example, for species 2 (0.1, 0.7, 0.1, 0.1) $E = 0.64$, and those dominated by pairs of species $E = 0.88$.

Table 1
Plant species used in the experimental designs at the three sites.

	Johnstown Castle	Moorepark	Athenry
Grass 1[a]	*Lolium perenne*	*Lolium perenne*	*Lolium perenne*
Grass 2[b]	*Dactylis glomerata*	*Phleum pratense*	*Phleum pratense*
Legume 1[a]	*Trifolium pratens*	*Trifolium repens*	*Trifolium repens*
Legume 2[b]	*Trifolium repens*	*Trifolium ambiguum*	*Trifolium ambiguum*

[a] Relatively fast growing grass or legume.
[b] Relatively slow growing grass and legume.

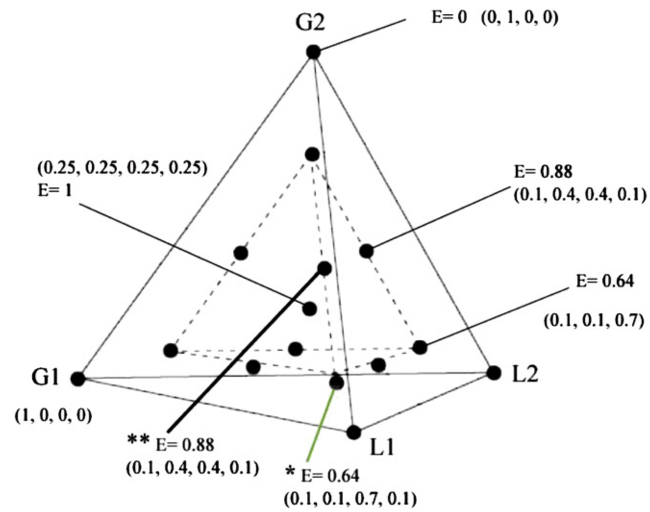

Fig. 1. The simplex design showing the proportions for four plant species (after Kirwan et al., 2007).

The monoculture and mixture communities were sown at two densities: low (12 kg sown viable seeds per hectare) and high (20 kg viable seed per hectare). The plots were also fertilised with compound nitrogen inputs at rates of either 100 kg ha^{-1} y^{-1} (low nitrogen) or 200 kg ha^{-1} y^{-1} (high nitrogen). Therefore, the complete design repeats the whole simplex four times, at two levels of density (overall sown abundance) and at two levels of nitrogen input.

The experiment was repeated at three sites in Ireland. Earthworms were sampled from 36 plots at each site. These were the four monocultures, the plot with equal proportions of each species and the four mixtures containing 70% of one species and 10% of each of the other three species, i.e. nine mixtures which were repeated at high and low nitrogen additions and high and low planting density. There was one replicate per treatment. The simplex design methodology does not require replication in the normal sense of the word because the analysis is by multiple regression with the proportions of the species and the rate of application of nitrogen and planting densities as the driving variables (Cornell, 1990).

2.2. Study area

The experimental plots were established within the framework of the European COST 852 Agro-Diversity Experiment. All the sites in Ireland were within Teagasc centres and were at Johnstown Castle (52° 16′ north, 6° 30′ west) (County Wexford), Moorepark (42° 8′ north, 8° 16′ west) (Co. Cork) and Athenry (53° 17′ north, 8° 44′ west) (Co. Galway).

The locations of the treatments within the site were determined randomly. The plot size was 16 m^2 at Johnstown Castle and 10 m^2 for Moorepark and Athenry. The soil characteristics are given in Table 2. The sites had previously been in permanent pasture. Before establishing the treatments they were sprayed with glyphosate to

Table 2
Geographical and soil characteristics for the four sites examined.

	Johnstown Castle (JC)	Moorepark (MO)	Athenry (AT)
Location	52°16′N, 6°30′W	42°8′N, 8°16′W	53°17′N, 8°44′W
Plot size	16 m^2	10 m^2	10 m^2
Soil texture	Sandy loam	Sandy loam	Stony to silt loam
pH	5.3	6.5	6.2