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Earthworm communities under boreal grass and legume bioenergy crops in pure stands and mixtures

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ABSTRACT

Crop mixtures can impact soil properties through their effect on earthworm communities. Earthworm abundance and species number in a bioenergy crop mixture can therefore provide valuable information about its stability and sustainability. In the context of developing sustainable bioenergy farming methods, this experiment aimed to determine the effects of pure stands of reed canary grass (*Phalaris arundinacea*) (RCG), the legume forage galega (*Galega orientalis*) and their mixture on earthworm populations, with bare fallow controls. Twice in each growing season in 2011 and 2012, soil blocks 25 cm × 25 cm × 25 cm were extracted from each plot, earthworms were manually separated from the soil and mustard oil was used to extract those from soil underlying the block. Four earthworm species were collected: *Aporrectodea caliginosa*, *A. rosea*, *Lumbricus rubellus*, and *L. terrestris*. Earthworm abundance ranged from no individuals in bare fallow in summer 2011 to 364 individuals m^{-2} in galega in spring 2012. Biomass of the worms ranged from 0 to 188 g m^{-2} and had the same trend as abundance. Of the cropped treatments, pure galega supported the greatest earthworm numbers and biomass, although being significant only in summer. Treatment effects were weak in most sampling periods but differed between seasons and this should be investigated in future studies. Our results demonstrate that specific plant species can have positive effects on the earthworm community under certain environmental conditions, which should be considered in bioenergy cropping.

Introduction

Much attention has been focused on perennial grasses as bioenergy crops because of their perceived benefits, such as low establishment and maintenance costs, and the potential to supply plentiful biomass feedstock (Larson 2008). Reed canary grass (*Phalaris arundinacea* L.) (RCG) is well established as an energy grass in Finland (Lewandowski et al. 2003). Most energy grasses are grown in monocultures, and often supplied with large inputs of synthetic nitrogen fertilizers. The use of monocultures in intensive agriculture is considered a major threat to farmland biodiversity (Hole et al. 2005), and is alleged to have reduced plant and soil invertebrate abundance and diversity in Europe (Preston et al. 2002). Earthworms are an important group of soil invertebrates exposed to and affected by this threat. Mixed cropping, the simultaneous growing of two or more plant species on the same land, can have an impact on earthworm communities which serve as key bio-indicators of soil quality (Doube and Schmidt 1997). A positive effect on the abundance and species number of earthworms can thus provide valuable information about the stability and sustainability of a cropping system (Bartlett et al. 2010).

Plant species diversity remains the most important plant community property affecting soil fauna populations (Eisenhauer et al. 2011a). Earlier studies have shown a positive relationship between plant species diversity and earthworm species number and abundance (Spehn et al. 2000), particularly when legumes are involved (Milcu et al. 2008). Different processes explain this relationship. The quality and quantity of plant derived residues made available to soil decomposers and herbivores in turn influences other soil fauna of the soil food web (Hooper et al. 2000; Eisenhauer et al. 2010). Plant species may also affect soil biotic community structure by differences in nutrient uptake (Grime 1994), differences in root exudates (Lavelle et al. 1995), increases in substrate diversity (Spehn et al. 2000), microhabitat modification (Eisenhauer et al. 2011b), and population fluctuations (Roscher et al. 2011). Furthermore, plant species mixtures affect soil physical properties (Niklaus et al. 2007), nutrient acquisition (Karanika et al. 2007) and soil C and N

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accumulation (Fornara and Tilman 2008). However, there is still relatively little information on the relationship between aboveground plant diversity and earthworm population structure.

Legumes, with their attendant rhizobia, have the ability to symbiotically fix N_2, therefore reducing or eliminating the need for synthetic fertilizer and otherwise contributing to the quality of the soil (Peoples et al. 2009). N-rich soils under legumes attracted more diverse soil fauna communities (Manhaes et al. 2013) and earthworms have responded positively to legume presence in earlier studies (Shipitalo et al. 1988; Gastine et al. 2003; Eisenhauer et al. 2009). Complementary effects have been shown between legume and non-legume species (Tilman et al. 1997), and earthworm populations were larger and more diverse in tropical and temperate grass–legume mixtures than in pure stands of the grass (Zou and Bashkin 1998; Schmidt et al. 2003). Earthworm populations generally show a strong negative response to fertilizers and pesticides (Lovell et al. 1995; Dinter et al. 2013), so more earthworms have been found in organic than conventional farms (Peres et al. 1998; Carey et al. 2009). Tillage has shown negative effects on earthworm communities in Finland (Nieminen et al. 2011).

To investigate the potential for sustainable energy cropping, we established an experiment where RCG was grown in various mixtures, and preliminary studies suggested that fodder galega or goat's rue (*Galega orientalis* Lam.) would be appropriate. As part of this project, we aimed at determining the effects of the two species, alone and in combination, on earthworm population structure.

Materials and methods

Experimental structure

The experiment was established in spring 2008 on loamy clay soil at the Viikki Research and Experimental Farm of the University of Helsinki (60°13′ N, 25°2′ E, 4 m amsl), Finland. The cropping history of the site was 5 years of continuous cereals, except for linseed in 2006, and mouldboard ploughing was regularly used. The soil is classified as a gleyic stagnosol (drainic), with topsoil pH of 6.1, bulk density of 1.08 g cm^{-3} and 35–42% clay content. The trial was comprised of 2.5 m × 16 m plots arranged in a randomized complete block design comprising eight treatments in four replicates. Four treatments were selected for this study, namely bare fallow, pure stand of RCG sown at 15 kg ha^{-1}, pure stand of galega sown at 12 kg ha^{-1}, and a 75:25 (seed ratio) mixture of RCG and galega. Galega seeds were inoculated with *Rhizobium galegae* (Elomestari Oy, Finland) before planting. Pure grass plots were fertilized each spring with 60 kg ha^{-1} of N (N-P-K 27-0-1; Yara Bela Suomensalpietari, Yara Oy, Finland). During this experiment, fertilization dates were 7 May 2011 and 30 April 2012. Legume-containing plots were given 20 kg ha^{-1} of N at sowing, and no further fertilizer. Any necessary weeding, including that of the bare fallow plots, was done manually in June.

The 30-year mean meteorological data (Fig. 1) were obtained from the Finnish Meteorological Institute station at Kaisaniemi, 9 km from the experimental site. Mean air temperatures were above average in July 2011, December 2011 and November 2012, but below average in February 2011 and December 2012. August and September 2011, April and May 2012 and September 2012 were much wetter than average.

Soil sampling and analysis

In July 2011 and May 2012, soil samples were collected from the topsoil (0–20 cm depth). From each plot, three samples were taken into steel cylinders and pooled for a composite sample in plastic bags and were then stored in freezer at −20 °C until analysis. About 500 mg dried, ground soil samples were used to measure total organic carbon and nitrogen by burning in a Vario MAX CN (Elementar Analysensysteme GmbH, Hanau, Germany). The ratio of C to N was calculated. Since organic matter concentration is difficult to measure directly in laboratories, the total organic carbon (%) was then multiplied by a conversion factor of 1.732 to convert it to organic matter concentration. This conversion factor assumes organic matter contains 58% organic carbon.

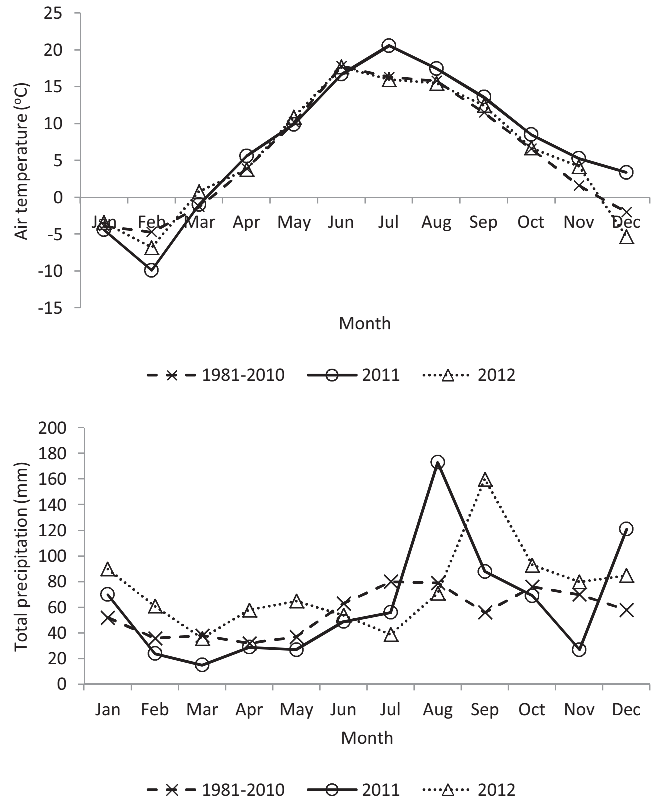

Fig. 1. Precipitation and air temperature for Helsinki in 2011 and 2012 and 30 years average.

Earthworm sampling procedure

Earthworm communities were sampled from the 16 plots four times over four seasons, on 26 July 2011 (80 days after fertilization) for summer 2011, 24 October 2011 for autumn 2011, 5 May 2012 (5 days after fertilization) for spring 2012 and 24 October 2012 for autumn 2012. The mid-summer sampling time had been recommended for lower latitudes, but was replaced by a spring sampling in the second year, while the autumn sampling time was chosen to capture the effects of the summer growing period. With the absence of buffers around each treatment to separate them, sampling sites were chosen in the centre rows, 1–2 m away from the end of each plot, and 7 m apart between and within plots during each sampling date, 3 m short of the recommended minimum distance of 10 m (Stoddard and Williams 2011). After surface vegetation was removed, a soil block (25 cm × 25 cm × 25 cm) was dug out and placed onto a large white plastic sheet. Earthworms were extracted by careful hand sorting, kept in wet paper towels and transported in labelled containers to the laboratory. A mustard-oil method (Gunn 1992; Gundale et al. 2005) was used to extract earthworms from soil underlying the block. An aliquot of 2 ml of mustard oil (allyl isothiocyanate) was added to 40 ml of isopropanol and diluted to 20 l with water. About 2 l of the solution was poured into the base of each hole and observed until earthworms ceased to