



# Effect of individual grass species and grass species mixtures on soil quality as related to root biomass and grass yield

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## ABSTRACT

For the purpose of feeding value, drought resistance and nitrogen utilization, other grasses (e.g. *Festuca arundinacea* and *Dactylis glomerata*) than the currently widely used perennial rye grass (*Lolium perenne*) are introduced in dairy farming, either as a monoculture or in a mixture. To study the effect of these grasses on yield and soil chemical and biological quality, the three species were sown in a field experiment in monoculture and in two mixtures. Within two growing seasons, the grass species tested under high soil fertility conditions did not show significant effects on most of the tested soil biological parameters. Only for the mixture of *L. perenne* and *D. glomerata* a higher soil  $\text{NO}_3^-$  and mineral N content were most probably related to a higher bacterial activity, possibly induced by dying roots of *L. perenne*. This was the likely cause of the high aboveground dry matter yield of this mixture. The N-efficiencies of the monocultures of *L. perenne*, *F. arundinacea* and *D. glomerata* were not different when only considering the aboveground biomass. In *L. perenne* and *F. arundinacea* the total N in root biomass was higher while under *D. glomerata* the  $\text{NO}_3^-$  in the soil was higher. The lower fraction of mineral N to total N for *L. perenne*, *F. arundinacea* and the mixture of the two suggests that their organic matter build-up/mineralization ratio was higher than for *D. glomerata*. Furthermore, the mixture of *L. perenne* and *F. arundinacea* showed significantly lower soil mineral N levels than the monocultures of each. We conclude that grassland systems with a mixture of *L. perenne* and *F. arundinacea* are more sustainable than the monocultures of each, in terms of reduction of nitrogen losses and the build-up of soil organic matter. *D. glomerata* should only be used in a mixture in which the companion grass(es) are maintained.

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## 1. Introduction

Organic farming and reduced use of external inputs, such as fertilizers and pesticides, implies a greater reliance on self-regulating ecosystem processes (Brussaard et al., 2007). Soil biota (soil organisms and plant roots) plays a key role in these processes. Grassland management directly and indirectly influences the soil biota and their functions (Bardgett, 2005). To develop and optimize sustainable grassland systems, insight is needed into how grassland management influences the soil quality in general and soil biota in particular (Van Eekeren et al., 2007).

For the purpose of specific feeding value (e.g. structural components) and drought resistance, other grasses (e.g. *Festuca arundinacea* and *Dactylis glomerata* L.) than the currently used perennial rye grass (*Lolium perenne* L.) are being introduced on dairy farms. Introduction can be either as a monoculture or in a

mixture with *L. perenne*. Some studies provide evidence that mixtures can improve grassland productivity (Remison and Snaydon, 1980b; Wilson and Newman, 1987) because the species differ in limiting growth factors, growing periods, and root characteristics (Whittington and O'Brien, 1968; Wilson, 1988). Especially complementary root characteristics in a mixture might develop a 'rootscape' that can exploit the soil volume more efficiently for water and nutrients than monocultures of each of the species (Crush et al., 2005).

Plants affect soil chemical and biological quality through various mechanisms: (1) the quantity and quality of resources allocated to the soil, (2) the extent to which plant species deplete nutrients and water from soils, and (3) the modification and formation of habitats for soil biota (Clement and Williams, 1964, 1967; Klein et al., 1988; Stone and Buttery, 1988; Carter et al., 1994; Wardle, 2002). Since the major source of organic matter in soil is from plant primary production, entering soil as litter-fall, root die-off and rhizodeposition, there is a tight coupling between plant and microbial productivity (Paterson and Sim, 2000). Different functional types of plants give rise to differences in microbial biomass in soils, as well as in micro-

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bial activity and community structure, as has been demonstrated in microcosms (Wardle & Nicholson, 1996; Graystone et al., 1998; Innes et al., 2004) as well as in the field (Groffman et al., 1996; Wardle et al., 1999). Similarly, nematodes differ in abundance and community structure in soils on which different functional types of plants are grown (Griffiths et al., 1992; Vikiotof et al., 2005).

In grasslands, productivity and soil quality are related to root characteristics. Wardle et al. (1997) suggest that the above-ground effect of plant litter on soil biota is of less importance than that of plant parts belowground. However, effects of plant species on soil biota are generally studied in the upper soil layer, while grass species are likely to differ in root properties at different depths. As a consequence, the effects of grass species on soil biota may differ accordingly between soil layers.

We conducted a field experiment with three grass species used in production grasslands: *Lolium perenne* (English rye grass), *F. arundinacea* (Tall fescue), *D. glomerata* (Cocksfoot) and *L. perenne* mixed with either *F. arundinacea* or *D. glomerata*. We measured root biomass, grass yield, and soil chemical and biological parameters at three soil depths; 0–10, 10–20 and 20–30 cm. The objectives of this study were (1) to assess the effect of grass species and mixtures on soil chemical and biological quality, (2) to quantify the effect of root biomass on soil quality, and (3) to measure the effect of grass species and mixtures on grass yield. We hypothesised that grasses with the highest root biomass would have the highest soil content of organic C and total N, and that this will be reflected in highest microbial biomass and abundance of nematodes. Furthermore, we hypothesised that mixtures of grasses would have a higher grass yield than that of the highest yielding monoculture.

## 2. Materials and methods

### 2.1. Study site and experimental design

This study was carried out from April 2007 to September 2008 on a grassland on sandy soil (Typic Haploquod according to USDA classification) in the South of the Netherlands (51°39'N, 5°11'E). Five treatments were established in a complete randomized block design with three replicates. The individual plot size was 24 m<sup>2</sup> (3 m × 8 m). The grass treatments were:

LP	<i>Lolium perenne</i> (cv. Bargala);
FA	<i>Festuca arundinacea</i> (cv. Barolex);
DG	<i>Dactylis glomerata</i> (cv. Ambassador);
LP/FA	mixture of <i>L. perenne</i> and <i>F. arundinacea</i> ;
LP/DG	mixture of <i>L. perenne</i> and <i>D. glomerata</i> .

Prior to the experiment, the field was in use as continuous maize land for a minimum of 10 years. Before sowing the grass treatments, dolomitic lime was applied at the rate of 2500 kg ha<sup>-1</sup>. In 2008 all plots received 250 kg N ha<sup>-1</sup> from inorganic fertilizer (calcium ammonium nitrate 27%) and 232 kg N total ha<sup>-1</sup> from organic fertilizer (dairy manure slurry).

### 2.2. Soil parameters

On 8 September 2008, two growing seasons after the start of the experiment, soil samples for determination of soil chemical and biological parameters were taken. Bulk samples of 70 cores (Ø 2.5 cm) at three soil depths (0–10, 10–20, 20–30 cm) were collected per experimental plot, sieved through 1 cm mesh, homogenized and stored at field moisture content at 4 °C before analyses. Sub-samples were taken for chemical, microbial and nematode analyses.

#### 2.2.1. Soil chemical parameters

Prior to further chemical analysis, samples were oven-dried at 40 °C. Soil dry matter content was determined after oven-drying of approximately 30 g of the bulk sample (in duplicate) at 105 °C. Soil acidity of the oven-dried samples was measured in 1 M KCl (pH-KCl). Organic C was measured by incineration of dry material at 1150 °C, after which the produced CO<sub>2</sub> was determined by an infrared detector (LECO Corporation, St. Joseph, Mich., USA). For determination of total N, gasses after incineration were reduced to N<sub>2</sub> and detected with a thermal-conductivity detector (LECO Corporation, St. Joseph, Mich., USA).

#### 2.2.2. Soil microbiological parameters

From the bulk soil sample, a sub-sample of 200 g field-moist soil was adjusted to 50% WHC (Water Holding Capacity) and pre-incubated at 12 °C for 4 weeks, to avoid the effects of temperature and moisture fluctuations in the field and to stabilize soil conditions (Bloem et al., 2006). After pre-incubation bacterial biomass was determined for all samples. Fungal biomass and bacterial growth rate was only determined for the treatments LP, DG and LP/DG while bacterial biomass was determined for all treatments. Microbial soil smears were prepared and measured as described by Bloem and Vos (2004). Fungal hyphae were measured using the grid-intersection method. Bacterial numbers and cell volumes were measured by confocal laser scanning microscopy and automatic image analysis (Bloem et al., 1995). Bacterial biomass was calculated from bacterial cell volume. Bacterial growth rate was determined as the incorporation of [<sup>3</sup>H]thymidine and [<sup>14</sup>C]leucine into bacterial DNA and proteins, respectively (Bloem and Bolhuis, 2006; Michel and Bloem, 1993). For a more detailed description, see De Vries et al. (2006).

Potentially mineralizable N was measured for the treatments LP, DG and LP/DG by anaerobic incubation of a soil sample under water (in slurry) for 1 week at 40 °C (Keeney and Nelson, 1982; Canali and Benedetti, 2006). These warm and anoxic conditions are optimal for a quick mineralization of organic matter by anaerobic bacteria. The lack of oxygen prevents conversion of released NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> (nitrification), so that N losses by denitrification cannot occur. The amount of mineral N (NH<sub>4</sub><sup>+</sup>-N) released is a measure of the quality (N content and decomposability) of the organic matter, and thus for biological soil fertility (Sparling and Schipper, 2002).

#### 2.2.3. Soil nematode parameters

Free-living nematodes were extracted from a sub-sample of 100 ml field-moist soil, using the Oostenbrink elutriator (Oostenbrink, 1960). Total numbers were counted and expressed per 100 g fresh soil. Nematodes were fixed in hot formaldehyde 4%, and at least 150 randomly selected nematodes from each sample were identified to genus and, whenever possible, to species. Nematode genera and species were assigned to trophic groups, following Yeates et al. (1993), and to colonizer-persister groups (cp-groups), according to Bongers (1990) and Bongers et al. (1995). The Nematode Channel Ratio (NCR) was calculated to express the relative contributions of bacterivorous (B) and fungivorous (F) nematodes to the total microbivorous nematode abundance (NCR = B/(B + F)) (Yeates, 2003). The Maturity Index was calculated as the weighted mean of the individual cp-values, in accordance with Bongers (1990) and Korthals et al. (1996). The Maturity Index is an ecological measure which indicates the condition of an ecosystem based on nematode species composition.

### 2.3. Crop parameters

The plots were harvested four times in 2007 and five times in 2008. Grass dry matter yield in the harvests was determined by cutting a strip of 0.84 m × 5 m with a two-wheel tractor. After weighing

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