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# Soil biota in grassland, its ecosystem services and the impact of management

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## Abstract

In the search for sustainable grassland systems, self-regulating processes in the soil become increasingly important. Soil biota play an important role in these processes and in the provision of various ecosystem services. For grassland systems important ecosystem services are supply of nutrients, soil structure maintenance and water retention. For developing and optimising sustainable grassland systems, insight is needed into the mechanisms by which soil biota are influenced by management and what it means for the functioning of the soil-plant system. Interactions between soil and plants can be represented by a cyclic conceptual framework including plant/roots, soil biota and soil properties. The challenge for sustainable grassland is to allow this cycle to function optimally with a minimum of external inputs. In these systems the soil food web is probably bacterial-based with a high density of earthworms. The impacts of grassland management on soil biota are discussed on the basis of two cases: use of grass-clover mixtures and a ley-arable crop rotation versus permanent grassland and continuous arable land.

Keywords: grassland, management, soil biota, ecosystem services

## Introduction

In intensive grassland systems, the importance of soil organisms has often been ignored, as physical manipulation of the soil and nutrient supply have been increasingly provided by human inputs rather than by natural processes (Brussaard *et al.*, 1997). In the search for sustainable agricultural grassland systems, self-regulating processes in the soil become more and more important (Yeates *et al.* 1997). Soil biota play an important role in these processes and in the provision of various ecosystem services: supply of nutrients, maintenance of soil structure, water regulation and, more generally the resistance and resilience of the belowground system (Brussaard *et al.*, 1997; Mulder *et al.*, 2006). For developing and optimising sustainable grassland systems, insight is needed into the mechanisms by which soil biota are influenced by management and what these mean for the functioning of the plant-soil system (Bardgett, 1996). This would allow us to make better use of the ecosystem services provided by soil biota and compensate for grassland systems that are developed in one direction (e.g. nitrogen use efficiency), with possible adverse effects on soil biota. This paper aims to outline the causal relations between grassland management, soil food web and ecosystem services. Therefore first information on the soil food web and ecosystem services will be reviewed briefly. Subsequently a conceptual framework is proposed in regard to cyclic plant-soil interactions under a grassland system. The application of this concept is shown by means of two examples of management measures: the use of grass-clover mixtures and a ley-arable crop rotation versus permanent grassland and continuous arable land.

## Soil biota

The living biomass of soil biota on conventional dairy farms in the Netherlands is on average 2656 kg soil biota ha<sup>-1</sup> in sandy soils and 3908 kg soil biota ha<sup>-1</sup> in clayey soils (Schouten *et al.*, 2000; Schouten *et al.*, 2002). These quantities exceed the weight of the livestock kept aboveground on these grasslands. According to Schouten *et al.* (2000; 2002) the live biomass of soil biota in sandy soils consisted of 81% bacteria and 13% earthworms and in clay soil data were 77% and 22% respectively. The remainder was made up of enchytraeids, mites, collembola and nematodes. Fungi and protozoa were not measured. However, recent analysis of organic dairy farms on sandy soils demonstrated that fungi contribute to 22 % of the total microbial biomass (De Vries *et al.* 2007).

Table 1. Soil biota in the soil (layer 0-10 cm, 0-15 cm earthworms) of 19 conventional dairy farms on sandy soil and 20 on clayey soil (Schouten *et al.*, 2000; Schouten *et al.*, 2002)

Classification	Biota	Units	Sand (n=19)	Clay (n=20)
Microflora	Bacteria	$\mu\text{g C g dry soil}^{-1}$	169	233
	Fungi		Not analysed	
Microfauna <200 $\mu\text{m}$	Protozoa	$\text{n } 100\text{g soil}^{-1}$	Not analysed	
	Nematodes		5464	4629
Mesofauna 100 $\mu\text{m}$ -2mm	Mites+Collembola	$\text{n m}^{-2}$	39722	37900
	Enchytraeids	$\text{n m}^{-2}$	17877	24908
Macrofauna > 2mm	Earthworms	$\text{n m}^{-2}$	148	318

Size relationships play an important role in biological interactions in soil, because the habitat is composed of differently-sized pores (Brussaard *et al.*, 1997). Soil biota can be classified by means of body width into microfauna, mesofauna and macrofauna (Table 1). In regard to feeding relations soil biota can be classified in functional groups. The interrelationships of the different functional groups are complex and can be seen more clearly in the context of the food chains or the soil food web (De Ruiter, 1993).

## Ecosystem services of soil biota

Important ecosystem services of soil biota for grassland systems are nutrient cycling, soil structure maintenance and water regulation. In grassland, the focus is on soil structure maintenance and water regulation, because of the perennial nature of the crop with no regular cultivation coupled with the compaction from animal trampling and tractor usage. In this paragraph, the contribution of microflora, nematodes and earthworms to these three ecosystem services in grassland is reviewed.

### Nutrient cycling

For the ecosystem service of nutrient cycling all groups of soil biota are involved. Bacteria and fungi directly govern this service, via nutrient mineralization and immobilisation. De Ruiter *et al.* (1993) estimated that the contribution by bacteria to N-mineralization is 20 to 140 kg N ha<sup>-1</sup> yr<sup>-1</sup>. The lower contribution by fungi to N-mineralization (1 to 24 kg N ha<sup>-1</sup> yr<sup>-1</sup>) is partly compensated for by a more efficient nutrient uptake of plants through the symbioses with mycorrhizal fungi. For example, Van der Heijden *et al.* (1998) measured positive effects on shoot phosphorous concentrations and shoot biomass of mycorrhizal species on grasses such as *Bromus* spp. and *Festuca* spp.

Nematodes and protozoa, affect nutrient cycling processes indirectly through the grazing of the soil microbial biomass and through excretion of nutrient rich waste. Griffiths (1989)

observed that the nitrogen content of ryegrass increased by 14% when nematodes or protozoa were added to microcosms with bacteria. Ingham *et al.* (1985) found increased grass and root growth when nematodes were added to a microcosm with bacteria. Not only microbivorous nematodes are involved in nutrient cycling. In experiments with clover, low levels of root infestation by clover cyst nematode (*Heterodera trifolii*) positively influenced the rhizosphere microbial community in the soil (Yeates *et al.*, 1998a; Denton *et al.*, 1999) and increased the root growth of white clover and perennial ryegrass with 141% and 219% respectively (Bardgett *et al.*, 1999a). Associated with this improvement in root production was a 322% increase in uptake of white clover derived <sup>15</sup>N. Similar results have been demonstrated for larger invertebrates, for example the larvae of the clover root weevil (*Sitona lepidus*) have been shown to facilitate the transfer of nitrogen from clovers to companion ryegrass (Murray and Hatch, 1994; Murray and Clements 1998).

Mesofauna and macrofauna affect nutrient cycling processes directly by fragmentation and transport of organic and mineral particles, and indirectly by regulating the microbial population and stimulating the microbial activity. Earthworms stimulate microflora in the structures that they create (Brown, 1995). Especially in grassland the function of fragmentation of organic matter by earthworms is important for breaking down the turf matt. Hoogerkamp *et al.* (1983) observed that, after the introduction of earthworms on reclaimed polder soils, in approximately three years a dark-coloured top soil started to develop. In an experiment of Clements *et al.* (1991) the most apparent effect of a pesticide treatment that excluded earthworms, was the accumulation of litter. Next to fragmentation, the transport and mixing of organic and mineral particles is an important function of earthworms. In a glasshouse study with perennial ryegrass and phosphate rock, it was shown that a treatment with earthworms had a higher yield, not only through the mixing of the phosphate rock but also by an increased extractability of P (Mackay *et al.*, 1982).

### Soil structure

In regard to the ecosystem service of soil structure maintenance, there is evidence that bacteria produce compounds that bind aggregates and that fungal hyphae attach particles to aggregates. Mäder *et al.* (2002) found in arable crops a positive correlation between aggregate stability and microbial biomass. Specific for mycorrhizal fungi, Tisdall and Oades (1979) measured under perennial ryegrass and white clover a positive relation between hyphal length and water stable aggregates.

Cole *et al.* (2006) concluded from their research at Sourhope that the macrofauna, particularly earthworms, has a more profound effect on the soil structure than the microflora. Earthworms affect soil structure through the production of faecal pellets, promotion of humification and creation of pores for movement of air, water and nutrients. An apparent example for the effect of earthworms on soil structure is the effect of artificially introduced earthworms in grasslands on reclaimed land (Hoogerkamp *et al.*, 1983). Here earthworm presence related to an increased infiltration capacity, an improved permeability and aeration of the upper soil layers and a considerable decrease in soil penetration resistance. Similar effects were reported in an experiment by Clements *et al.* (1991) in which the 20 years' absence of earthworm and other invertebrates, due to a pesticide treatment, increased soil bulk density, penetrability and reduced soil organic matter content, initial infiltration rate, pH and soil moisture content.

## Water regulation

The function of water regulation is closely linked to the above described functions of soil structure maintenance and nutrient cycling. For example, a better aggregate stability improves the water retention or through an improved nutrient intake of plants by mycorrhizal fungi, plants become less vulnerable to the first drought stress (Smith and Read, 1997). An example of the effect of soil biota on water regulation is the increased water logging in Scottish grasslands due to the flatworm predation on earthworms (Haria *et al.*, 1998). Water infiltration through burrows and stable crumb formation are two key soil factors strongly affected by earthworms. Bouché and Al-Addan (1997) correlated infiltration rate to earthworm biomass and calculated a mean rate of 150 mm water h<sup>-1</sup> per 100 g m<sup>-2</sup> earthworms. Earthworm activity may attribute to improvement of infiltration resulting in a reduced runoff. However, these effects on macropore formation can also lead to an increased bypass flow and greater leaching of nutrients. (Edwards *et al.*, 1992).

The rooting depth of grass roots is especially important for the drought resistance of grassland. Earthworm burrows create pathways for the penetration of roots through the soil. Channels of the deep-burrowing, surface-feeding earthworms (*Lumbricus terrestris* and *Aporrectodea longa*) are generally vertical and may penetrate a hard pan. In several pot and field studies increased root growth has been measured in response to earthworm inoculation (Logsdon and Linden, 1992).

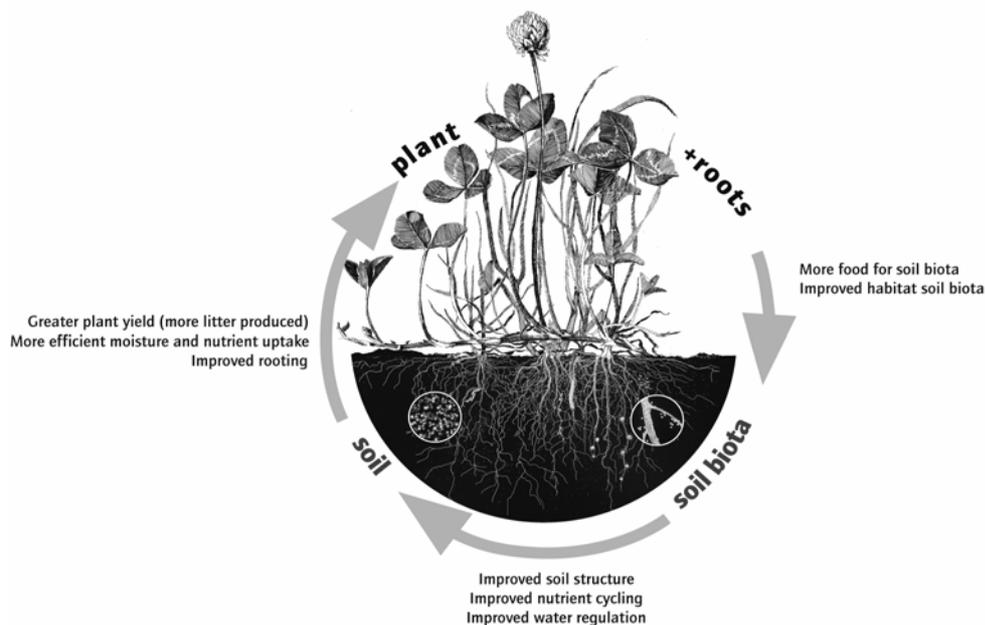
## **Plant-soil interactions**

The influence of grassland management on soil biota can not be seen apart from effects on the grass sward. There is an intimate link between soil organisms and the grass plant. In this paragraph different plant-soil interactions are illustrated and elaborated in a conceptual framework.

Through root exudates and litter from roots and aboveground parts, grasses supply nutrients to the soil food web. Deinum (1985) calculated that from a perennial ryegrass sward 4500 kg organic root mass ha<sup>-1</sup> is decomposed on a yearly basis. According to Whipps (1990) approximately 35-80% of the net fixed C in perennial grasses is transferred below ground. The quantity and quality (including plant secondary metabolites) of root exudates and plant litter are the major factors which determine the soil food web and its diversity under a specific plant species and even different grass species (Bardgett, 2005). These effects can be illustrated with various experiments in which was shown that single species of grassland plants differed markedly in their impact on soil microbes (Bardgett *et al.*, 1999b), nematodes (Wasilewska, 1995) and enchytraeids (Griffiths *et al.*, 1992). Next to a source of food, grass roots are a host for many soil organisms such as root pathogens, root herbivores, and symbiotic soil biota. Therefore the rhizosphere is one of the “hot spots” for biological activity (Beare, 1995). In turn, soil biota, influence grass production and quality through their ecosystem services and antagonistic relations from root herbivores. By means of these processes, the soil biota also influence the composition of grassland plant communities. For example Grime *et al.* (1987) showed in a microcosm experiment, that the presence of mycorrhizal fungi lead to a shift in plant composition.

With this the circle is accomplished; plants influence, through the quantity and quality of root exudates and litter, the soil biota and its diversity. The soil biota in turn influence with their ecosystem services and antagonistic relations, the plant production, plant quality and diversity

of the plant community. In regard to earthworms, Syers and Springett (1983) concluded that these plant-soil relationships concerning earthworms are interactive, cyclical and complex. Bardgett and Wardle (2003) elaborated the links between grazers, plants and soil for the decomposer organism. An illustrative example for these links is revealed by an experiment of Hamilton and Frank (2001) in which grazing of *Poa pratensis* promoted root exudation. This stimulated microbial biomass in the root zone which in turn increased soil N availability and plant N acquisition, which resulted in grass growth. Brussaard (1998) combined the soil-plant interactions from “decomposers” in a diagram with the interactions of “root-biota” including the root herbivores and “ecosystem engineers” including the earthworms. In this diagram Brussaard (1998) considered plant roots as ecosystem engineers since they create habitats for other organisms. A conceptual framework is proposed in which the diagram of Brussaard (1998) is worked out in a cyclical process according to Syers and Springett (1983) for a grassland sward (Figure 1). Through plants and roots, and interactions with soil biota, food and habitat for biota is delivered to the soil. Through the improvement of nutrient cycling, soil structure and water retention by soil biota, plant rooting is increased and the intake of nutrient and water through plants is increased. Finally this results in a greater plant yield which means again an increase in litter and root exudates in quantity and quality. The challenge for sustainable grassland management is to allow this cycle to function optimally with a minimum of external inputs. It can be compared to cranking up of an engine, once the flywheel is turning, the engine can continue at a low speed with minimal inputs and even sustain minor disturbances. For a grassland the grassroots are a major link between the aboveground and belowground system. When the roots fail to grow the cycle shuts down.



Figur 1. Cyclic interactions between plant/roots, soil biota (root biota, decomposers and ecosystem engineers) and soil properties (chemical and physical).

In agreement with Wardle *et al.* (2004) it is assumed that the bacterial-based soil food web with a high density of earthworms, delivers the ecosystem services that go together with the optimal functioning of these plant-soil cycle. More precisely this means a fast nutrient cycling process, maintenance of soil structure and water regulation. In the comparison of ecological interactions between aboveground and belowground biota, Wardle *et al.* (2004) make a distinction between fertile systems that support high herbivory and infertile habitats that support low herbivory. Ecosystems dominated with plant species adapted to fertile conditions

('mull sites'), have a high litter quantity and quality, and support soil food webs with a bacterial-based energy channel, microfauna (nematodes and protozoa) and a high density of earthworms. On the contrary infertile soils ('mor sites'), support plants with low litter quantity and quality, and tend to support soil food webs dominated by fungi and arthropods such as mites and collembolans (Wardle, 2002). The interaction between the aboveground and belowground biota in the infertile, unproductive ecosystems are negative and in the fertile, productive ecosystems positive (Wardle *et al.*, 2004). These positive interactions are just the cyclic aspects needed for a sustainable agricultural grassland.

### **Impact of grassland management on soil biota and its services**

In this paragraph the effect of two common grassland management measures on soil biota and its services are discussed. Management affects soil biota directly (e.g. via soil qualities) and indirectly (e.g. via the cycle plant-soil interactions). For example in an upland grassland in the Scottish Borders, lime application had both direct effects on the enchytraeid community structure by soil chemical qualities as well as on the nematode community structure by an increased plant production and root turn-over (Cole *et al.*, 2006; Murray *et al.* 2006).

#### Use of grass-clover mixtures

The primary motive to use clover is its ability for N-fixation, in order to reduce the reliance on artificial fertilizer. However, indirectly white clover possibly alters the soil biota through the quality and quantity of litter and root exudates. At one side the root density of white clover is considerably lower than from grass (Evans, 1977; Tisdal and Oades, 1979; Schortemeyer *et al.*, 1997). This suggests that the quantity of litter from roots is lower. On the other hand the litter quality of white clover is better than grass, because of a lower C/N ratio (Neergaard *et al.*, 2002). Leaf litter from a *Trifolium* species showed a higher Substrate Induced Respiration (SIR, a relative measure of active microbial biomass) compared to litter from a grass (Beare *et al.*, 1990). In a microcosm study, with comparable root weights for grass and white clover, a higher microbial biomass for clover was found (Mawdsly and Bardgett, 1997). Tisdal and Oades (1979) reported in a pot experiment that white clover roots had a higher infection with mycorrhiza fungi (50.8%) compared with ryegrass roots (13.3 %). However, because the ryegrass had eight times the length of root of white clover, the total length of infected root of ryegrass was twice that of white clover.

In a field study with pure grass and grass-white clover mixtures, De Vries *et al.* (2006) found a higher microbial biomass with a higher fungal:bacterial ratio in pure grass. Elgersma and Hassink (1997) determined no difference between microbial biomass under grass or grass-clover but the amount of active microbial biomass was higher under mixtures. Salamon *et al.* (2004) found at the Swiss BIODDEPTH site an increase in the number of collembola in the presence of legumes, benefiting from high litter quality and increased microbial biomass in the rhizosphere of these plants. In different field experiments, a higher biomass of earthworms was found in grass-clover mixtures compared to pure grass (Sears, 1950; Yeates *et al.*, 1998b; Baars, 2002; Van Eekeren *et al.*, 2005). Here also specific litter quantity can play a profound role.

What would the increase in the number of earthworms mean for the ecosystem services in the plant-soil cycle when clover is introduced? Possible this could enhance the plant-soil cycle with the function of soil structure maintenance and water regulations under grasslands. Mytton *et al.* (1993) tested the hypothesis that clover is more effective than ryegrass in

developing rapid improvements in soil structure. Significant differences in drainage rates supported the hypothesis whereas no differences in bulk density, porosity or aggregate stability between the treatments were found. Soil moisture characteristic curves indicated that a more free-draining-structure develops under clover than under grass due to a higher ratio of macro- to micropores.

In conclusion, the results suggest that introduction of white clover in a grass sward reduces the root density but due to the better litter quality, the differences in microflora between grass and grass-clover mixtures in the field are small. However, the biomass of earthworms seems to increase which has, together with other soil biota, a positive impact on the ecosystem service of water regulation. Herewith, the introduction of white clover can be a tool to enhance the cycle of plant-soil interactions.

#### Ley-arable crop rotation versus permanent grassland and permanent cropping

Conventional and organic dairy farms, with grass and maize, show an increasing interest for ley-arable crop rotations, because of an improved nitrogen use efficiency (Neuens and Reheul, 2002). Also improved conditions for clover as well as the opportunity for mechanical weed control are motives (Younie and Hermansen, 2000). However, what is the impact on soil biota, its services and the plant-soil cycle, when on farm level permanent grassland and continuous maize cropping is changed to ley-arable cropping?

The soil food web under permanent grassland is completely different from continuous arable land. Fromm *et al.* (1993) showed that the type of land use (grassland versus arable land) even had more influence on soil biota than different soil types with the same land use. In the Dutch Soil Quality Monitoring Network the bacterial biomass was 50-100% higher in grassland than in arable land while the bacterial activity was higher in arable land (Bloem *et al.*, 2006). In the same study higher numbers of nematodes in grassland compared to arable land were measured (Schouten *et al.*, 2004). Juma and Mishra (1988) assessed a dominance of herbivorous nematodes under a perennial crop and bacterivorous nematodes under arable land. Different authors (Low, 1972; Yeates *et al.*, 1998b; Lamandé *et al.*, 2003) found earthworms to be more abundant under long-term pasture than under long-term cropping. Low (1972) reported that after 25 years of regular cultivation, the number of earthworms were only 11-16% of those in old grassland. An important explanation for the difference in soil food web under grassland and arable land is the availability in resources (e.g. total soil carbon and living roots). In addition to food supply, the mechanical damage and predation after cultivation play an important role, especially for meso- and macrofauna (Wardle, 1995). The difference in soil food web under grassland and arable land, influences the deliverance of ecosystem services supplied by the soil biota. Particularly the absence of earthworms in arable land has its impact on the services of soil structure maintenance and water regulation.

For a ley-arable crop rotation the following questions arise: How are the soil biota and its services restored in a grass ley after arable cropping, compared to a permanent grassland? How are these services, after restoration in the ley phase, conserved into the arable phase?

Concerning the recovery of microflora after tillage, Buckley and Schmidt (2001) found that the soil microbial community structure of an old arable field, 7 years after cultivation had been abandoned, still remained more similar to nearby cultivated sites than to fields which had never been cultivated. Similarly, Steenwerth *et al.* (2002) showed distinct differences in Phospholipid ester-linked fatty acid (PLFA) profiles of old permanent pastures compared to

profiles of fallow grasslands. They suggested that the soil environment and the associated microbial community may take decades to recover from cultivation effects. For nematodes, Villenave *et al.* (2001) determined that full restoration was not achieved within 10 years of fallow. Wasilewska (1994) showed that nematode taxa known to be colonizers or r-strategists dominated in younger meadows while persisters or K-strategists taxa dominated in older meadows. Yeates *et al.* (1998b) found that earthworm biomass increased after 5 years perennial pasture on sites formerly under arable cropping but not reached the level of permanent pastures. These data suggest that when cultivation is stopped and a grass fallow or grass ley is established changes in the soil biota composition takes place but restoration to the level of permanent grassland takes years.

Less information is available on the effect of ploughing a ley for arable land. Hatch *et al.* (2002) showed that the SIR and BIOLOG profiles of a ley resembled already the first year after cultivation those from arable land. For nematodes, Sohlenius and Sandor (1989) measured that it will take longer than one year before a ploughed ley resembles a continuous arable land. Concerning the functioning, Anderson and Domsch (1990) showed that the soil microbial biomass in a crop rotation had a more efficient respiration than from the microbial biomass in continuous cropping. This would suggest that, a ley-arable crop rotation could improve the ecosystem services of soil biota above continuous cropping.

In conclusion, results show that it takes years to restore the soil food web and its services in a grass ley to the level of permanent pasture. When the plant-soil cycle in a permanent pasture is running smoothly, ploughing up a permanent pasture for ley-farming should be avoided. A ley-arable crop rotation could benefit from ecosystem services of soil biota above continuous cropping. When a ley-arable crop rotation is practised, the length of the ley phase should be minimized. This, in order to reduce the pressure on permanent grassland on farm level and to overcome that lack of soil structure maintenance and water regulation by soil biota in the ley phase is decreasing the production. Since the number of earthworms is low at the start of the ley and restoration of the earthworms takes years, the services of soil structure maintenance and water regulation lag behind. In a young ley, this could possibly create difficulties in keeping the perennial cycle of plant-soil interactions running after the effects of soil cultivation on soil structure and water regulation are faded away. To stimulate these services earthworms could possibly be stimulated by sowing clover (Paragraph 5.1).

## Conclusions

For developing and optimising sustainable grassland systems, insight is needed into the mechanisms by which soil biota are influenced by management and what these mean for the functioning of the plant-soil system. The causal relations between grassland management, soil food web and its ecosystem services can be clarified in a cyclic conceptual framework with plant/roots, soil biota (root biota, decomposers and ecosystem engineers) and soil chemical properties (chemical and physical). The challenge for sustainable grassland management is to let this cycle function optimally with a minimum of external inputs. The basis of this cycle is a productive grassland with a good litter quantity and quality and a good functioning root system. It is assumed that the basal soil food web for sustainable agricultural grassland, is a bacterial-based food web taking care of a fast nutrient cycling process and a high density of earthworms taking care of, amongst others, the maintenance of soil structure and water regulation.

The introduction of white clover in a grass sward reduces the root density but due to the better litter quality, the differences in microflora between grass and grass-clover mixtures are variable. However, the biomass of earthworms seems to increase which has, together with other soil biota, a positive impact on the ecosystem service of water regulation. Herewith the introduction of white clover can be a tool to improve the cycle of plant-soil interactions.

For ley-farming, it takes years to restore the soil food web and its services in a grass ley to the level of permanent pasture. Ploughing up permanent pastures for ley-farming means a loss of soil biota and its services. A ley-arable crop rotation could benefit from ecosystem services of soil biota above continuous cropping. When a ley-arable crop rotation is practised, the ley phase should be only two to three years. This, in order to minimize the pressure on permanent grassland on farm level and to overcome that lack of soil structure maintenance and water regulation by soil biota in the ley phase is decreasing the production. To stimulate earthworms and their ecosystem services in the ley phase, a grass-clover mixture could be sown.

In the light of the plant-soil cycle, results show that use of clover is a sustainable measure but the introduction of ley-arable farming on a dairy farm should not be practised at the cost of permanent grasslands with its soil biota and its ecosystem services.

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