

## Effect of grass-clover on the ecosystem services soil structure maintenance and water regulation

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### Abstract

*White clover has a lower root biomass and a higher abundance of earthworms than grass. This might have an impact on the ecosystem services soil structure maintenance and water regulation when white clover is introduced in the grassland on organic dairy farms. We investigated the root biomass, the abundance of earthworms and a selection of soil physical parameters in white clover, grass-clover, and grass with and without N fertilizer. The treatment with clover-only had a lower root biomass, a lower C/N-ratio of the roots, a higher abundance of earthworms, a higher number of earthworm burrows, a lower penetration resistance at the 20-30 cm soil layer and a lower proportion of crumbs in the soil, than the other treatments. This confirms the literature that pure clover stimulates the ecosystem services of water regulation, but is less conducive to soil structure maintenance. However, the grass-clover mixture did not differ significantly from the grass treatments, but differed from pure clover in a higher percentage of soil crumbs. We infer that, when clover is introduced in grassland on organic dairy farms to fix atmospheric N<sub>2</sub>, the mixture of grass and clover maintains the positive impact of grass roots on soil structure but only may show a positive effect of clover-only on water regulation with a higher clover percentage in the dry matter.*

### Introduction

In sustainable grassland the focus is on ecosystem services like 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. For these ecosystem services, roots and soil biota play an important role. When sustainable grassland systems are developed it is important to know which effect management measures have on roots, soil biota and the functioning of the soil-plant system. One of the management measures on organic dairy farms is the introduction of white clover (*Trifolium repens*) with its ability to fix atmospheric N<sub>2</sub> in symbiosis with *Rhizobium* bacteria. However, it is well documented that the root density of white clover is considerably lower than that of grass (Robinson and Jacques, 1958). Since the organic material released by living or decomposing roots stabilizes aggregates directly or indirectly by providing nutrients to micro-organisms,

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the lower root density could have a direct impact on soil structure maintenance. Robinson and Jacques (1958) measured a lower percentage of stable soil aggregates in white clover than in perennial ryegrass. On the other hand, Sears (1950) found a higher earthworm biomass in a grass-clover mixture than in grass-only swards. Earthworms are known for their positive effect on soil structure and water regulation through their burrowing activity and earthworm burrows characteristics (Clements *et al.*, 1991). Mytton *et al.* (1993) found higher drainage rates in white clover than in perennial ryegrass. Altogether this would suggest that with the introduction of white clover in grassland, soil structure maintenance could deteriorate, while water regulation would improve. In the present field study, we measured the root biomass, the abundance of earthworms and a selection of soil physical parameters in white clover-only, a grass-clover mixture, and grass-only with and without N fertilizer. Our objectives were (1) to measure the effect of the treatments on the root biomass and abundance of earthworms, and (2) to explore the relevance of changes for the ecosystem services soil structure maintenance and water regulation.

## Methods

### *Sampling site and experimental design*

The experiment was established in spring 2004 on a free-draining sandy loam soil (7.2-7.5 % clay (< 2  $\mu\text{m}$ )) in the east of the Netherlands (52°26'N, 6°08'E). Four treatments were established in a completely randomized block design of six blocks: Grass with N fertilizer (GN1), Grass without N fertilizer (GN0), Grass-clover without N fertilizer (GCN0) and Clover without N fertilizer (CN0). In order to get approximately the same quantity and quality (C/N ratio) in the above- and below-ground biomass in GN1 and GCN0, inorganic fertilizer (calcium ammonium nitrate 27%) was applied on GN1 at a rate of 150 kg N ha<sup>-1</sup>. The percentage clover dry matter in 2005 was on average 26% for GCN0 and 75% for CN0.

### *Soil sampling and analysis*

On 16 December 2005, two growing seasons after the start of the experiment, soil samples were taken. Three soil cores (0-10 cm,  $\varnothing$  8.5 cm) per plot were taken to determine the root biomass. Only the 0-10 cm soil layer was measured since from other experiments is known that 75% of root biomass is concentrated in this layer (Van Eekeren *et al.*, 2010). After washing the roots for soil, the roots were oven-dried at 70 °C and the dry matter, ash content and total N of the roots was measured.

Earthworms were sampled in two blocks (20x20x20 cm) per plot. The earthworms in the blocks were hand-sorted counted and weighed. Before the blocks were sorted for earthworms, in one block per plot the earthworm burrows were counted on horizontal surfaces (20x20 cm) exposed at 10 cm and 20 cm depth.

Penetration resistance was measured with a penetrometer (Eijkelkamp, Giesbeek, The Netherlands) with a cone diameter of 2 cm<sup>2</sup> and a 60° apex angle. Soil structure was determined in 1 block (20x20x20 cm) per plot. The soil was divided by visual observation into crumbs, sub-angular blocky and angular blocky elements.

### *Statistical analysis*

The treatment effects on the measured parameters were tested using one-way ANOV, using the GENSTAT statistical software (8<sup>th</sup> Edition, Hemel Hempstead, UK)

## Results

CN0 had significantly lower grass root biomass and significantly higher clover root biomass than the other treatments (Table 1). The C/N ratio in the total root biomass was lowest for CN0 and highest for GN0. GN1 and GCN0 were intermediate. Earthworm abundance was significantly higher in CN0 than in the other treatments

(Table 1). Earthworm numbers and biomass were negatively correlated with the C/N ratio of the root biomass ( $r=-0.59$ ,  $P=0.002$  and  $r=-0.52$ ,  $P=0.01$ , respectively). The number of earthworm burrows at 10 cm depth was significantly higher in CN0 than in the other treatments. At 20 cm depth, the number of earthworm burrows was highest in the two treatments with clover (GCN0 and CN0), but it was not significant different from GN1. The number of burrows at 10 cm and 20 cm depth was positively correlated with the earthworm biomass ( $r=+0.50$ ,  $P=0.012$  and  $r=+0.49$ ,  $P=0.015$ , respectively). The penetration resistance in all soil layers was lower in clover-only (CN0) than in the grass-only with N fertilizer (GN1), but this was only statistically significant in the soil layer at 20-30 cm depth. The penetration resistance at 20-30 cm was negatively correlated with earthworm biomass ( $r=-0.47$ ,  $P=0.02$ ). The proportion of crumbs was significantly higher in GN0 than CN0 (Table 1). GN1 and GCN0 took an intermediate position. The CN0 had the highest proportion of angular blocky elements. The proportion of crumbs was negatively correlated with clover root biomass ( $r=-0.53$ ,  $P=0.008$ ), but no significant correlation was present with grass or total root biomass.

**Table 1. Root, earthworm and soil physical parameters in grass with added N fertilizer (GN1), grass without N fertilizer (GN0), grass-clover without N fertilizer (GCN0) and clover without N fertilizer (CN0).**

Parameter	Unit	Treatments				P-value
		GN1	GN0	GCN0	CN0	
<b>Root biomass 0-10 cm</b>						
Grass	G AFDM m-2	169a	217a	177a	12b	<0.001
Clover	G AFDM m-2	0c	1c	16b	62a	<0.001
Total	G AFDM m-2	169a	218a	193a	73b	<0.001
Total N	G N m-2	4.0a	4.1a	4.5a	2.6b	0.043
C/N		21.0b	26.3a	21.3b	14.2c	<0.001
<b>Earthworms</b>						
Total number	N m-2	322b	326b	359b	480a	0.002
Total biomass	G m-2	82b	76b	110ab	135a	0.009
<b>Earthworm burrows</b>						
10 cm depth	N m-2	58b	67b	138b	225a	0.002
20 cm depth	N m-2	50ab	8b	113a	121a	0.023
<b>Penetration resistance</b>						
0-10 cm	mPa	1.48	1.44	1.46	1.39	0.776
10-20 cm	mPa	1.46	1.45	1.40	1.34	0.368
20-30 cm	mPa	2.51a	2.39ab	2.45ab	2.13b	0.036
<b>Soil structure 0-10 cm</b>						
Crumb	%	39bc	53a	50ab	32c	0.006
Sub-angular	%	13	9	12	5	0.094
Angular	%	47b	38b	38b	62a	0.009

Values followed by the same letter within a row are not statistically different at the 5% error level for the main treatment effect.

## Discussion and conclusions

In line with other research (Robinson and Jacques, 1958), the root biomass in clover-only was less than in grass-only. However, the mixture of grass and clover had the same root biomass as grass-only. Although the soil structure was only correlated with clover root biomass and not with grass or total root biomass, the soil structure followed the same pattern; the soil structure in clover-only was less developed than in grass-only and the grass-clover mixture. Since the grass root mass and the soil structure in the grass-clover mixture were comparable with the grass-only treatments, we suggest that the soil structure of clover mixed with grass is maintained at the same level. Further research on soil aggregate stability is needed for confirmation. The earthworm biomass was higher (70%) in clover-only (CN0) than in grass-only (GN1 and GN0), with the mixture of grass and clover in an intermediate position. Sears (1950) found a higher earthworm biomass in a grass-clover mixture than in grass-only swards. Thus, introduction of clover in a grass sward results in higher earthworm population densities. The negative relationship between the C/N-ratio of the root biomass and the total abundance of earthworms, suggests that the quality of the litter rather than the quantity played a prominent role in the higher abundance of earthworms. Water regulation as an ecosystem service in grasslands is greatly influenced by earthworms and their burrows (Clements *et al.*, 1991; Edwards and Shipitalo, 1998). In our experiment, the numbers of earthworm burrows at 10 and 20 cm depth were highest in clover-only. Furthermore, clover-only showed the lowest penetration resistance at 20-30 cm, suggesting improved water infiltration. These data are consistent with results of Mytton *et al.* (1993), who found that white clover-only drained more rapidly than grass-only. For both drainage and soil moisture characteristics, Mytton *et al.* (1993) found that a grass-clover mixture (> 50% clover in the DM) took an intermediate position between the monocultures of grass and clover. In our research, the mixture of grass-clover (GCN0), with 26% clover in the DM, showed a higher number of earthworm burrows and a lower penetration resistance than grass-only with fertilization (GN1), but differences were not significant. This suggests that a positive effect of clover on water infiltration was not apparent in our grass-clover mixture. With a higher clover percentage in the dry matter this might be different.

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