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Ecosystem services in grassland associated with biotic and abiotic soil parameters

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ABSTRACT

Biotic soil parameters have so far seldom played a role in practical soil assessment and management of grasslands. However, the ongoing reduction of external inputs in agriculture would imply an increasing reliance on ecosystem self-regulating processes. Since soil biota play an important role in these processes and in the provision of ecosystem services, biological soil parameters should be an integral part of soil assessment. The general objective of the current study is to investigate to what extent biotic soil parameters provide additional value in soil quality assessment of grassland on sandy soils. We measured abiotic and biotic soil parameters together with process parameters underlying ecosystem services in 20 permanent production grasslands. Cross-validated stepwise regression was used to identify abiotic and biotic soil parameters that explained the soil ecosystem services soil structure maintenance, water regulation, supply of nutrients, and grassland production, respectively.

Process parameters underlying the ecosystem service soil structure maintenance such as bulk density and the percentage of sub-angular blocky elements were mainly influenced by SOM and its qualities. The correlations between penetration resistance at 0–10 cm and the percentage of soil crumbs with earthworms suggested a relationship to earthworm activity. Parameters underlying the service of water regulation showed no clear relationship to biotic soil parameters. Water infiltration rate in the field was explained by the penetration resistance at 10–20 cm. Process parameters underlying the service of nutrients' supply such as the potentially mineralizable C and N were mainly determined by soil total N. The potential C and N mineralization were more related to biotic soil parameters, whereby each parameter was the other's antithesis. The grassland production without N fertilization viz. the nitrogen supply capacity of the soil measured as N yield, was mainly explained by soil organic matter (SOM) and soil moisture, and to a lesser extent by soil total N. One gram of SOM per kg of dry soil corresponded to 3.21 kg N yield ha^{-1}, on top of a constant of 15.4 kg N ha^{-1}. The currently applied calculations in the Dutch grassland fertilization recommendation, underestimated in 85% of the production grasslands, the measured nitrogen supply capacity of the soil by on average 42 kg N ha^{-1} (31%). This legitimizes additional research to improve the currently applied recommendations for sandy soils. The response of N yield to N fertilization ranged from 35 to 102%. This wide range emphasizes the importance of a better recommendation base to target N fertilizer. The response of N yield to N fertilization was predicted by the total number of enchytraeids, the underlying mechanism of which needs further investigation on different soil types. This knowledge can be important for the optimal use of fertilizer and its consequences for environmental quality.

1. Introduction

Soil quality is globally acknowledged as the major factor determining yield and quality of crops. Although many definitions exist, agronomic soil quality can be defined as the sustained ability of

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a soil to (i) provide enough water and nutrients to crops, (ii) maximize the use efficiency of external inputs, (iii) minimize negative influences on the environment and (iv) sustain soil biodiversity. In The Netherlands, soil quality of production grasslands has not been a matter of concern in the last few decades. Nutrients and irrigation could be applied in abundance and could thus compensate for a lack, if any, of agricultural soil quality. Legislative restrictions have, however, reduced the use of organic and inorganic fertilizers (Vellinga, 2006), and irrigation. This has led to a renewed interest in the potential for optimizing yield and nutrient use efficiency by improving soil quality.

Soil quality can be assessed by parameters based on chemical, physical and biological properties. Soil quality of permanent grassland is generally assessed on the basis of a number of abiotic parameters (e.g. soil organic matter, total N, pH, K-HCl and P-Al). Biological soil properties have so far seldom played a role in practical soil assessment. However, the reduced use of external inputs implies a greater reliance on self-regulating processes (Brussaard et al., 2007). Soil biota play an important role in these processes and in the associated provision of various ecosystem services, such as supply of nutrients to plants, maintenance of soil structure, water regulation and grass production (Brussaard et al., 1997; Swift et al., 2004; Mulder, 2006; Kibblewhite et al., 2008). Therefore, biotic soil parameters could play a role in future soil assessment. Relationships between soil biota, soil ecosystem services including grass yield and/or soil quality, have been established in different microcosms and field experiments. Bacteria and fungi govern nutrient supply via nutrient mineralization and immobilization (De Ruiter et al., 1993). In regard to the service of soil structure maintenance, there is evidence that the polysaccharides produced by bacteria bind aggregates together, and that fungal hyphae entangle soil particles and smaller aggregates into larger aggregates (Tisdall and Oades, 1979; Six et al., 2002; Mäder et al., 2002).

A relationship between microbial biomass nitrogen and nitrogen uptake by grass was detected by Hassink (1995a). Protozoans, nematodes, Collembolans and mites affect nutrient cycling through grazing on micro-organisms and excretion of nutrients (Ingham et al., 1985; Griffiths, 1989; Bardgett and Chan, 1999; Vreeken-Buijs et al., 1996), and thus increase the N content and growth of grass (Ingham et al., 1985; Griffiths, 1989). Earthworms and enchytraeids increase nutrient cycling processes through fragmentation and mixing (Mackay et al., 1982; Clements et al., 1991; Brown, 1995; Cole et al., 2000; Mulder et al., 2006; Postma-Blaauw et al., 2006). Furthermore, they affect soil structure through the production of faecal pellets, promotion of humification and creation of pores (Hoogerkamp et al., 1983; Clements et al., 1991), and support water regulation through burrows, stable crumb formation and root growth stimulation (Logsdon and Linden, 1992; Bouché and Al-Addan, 1997; Haria, 1998). Introduction of earthworms has led to an increase in grass production (Stockdill, 1982; Hoogerkamp et al., 1983; Baker, 1998). Hence, it is well established that the soil biota play vital roles in the functioning of the ecosystem and associated ecosystem services, including grassland production.

To date, the observed relationships are virtually not translated into biotic soil indicators useful for soil quality assessment, although references to biotic soil parameters (Rutgers et al., 2009) and the effect of grassland management on soil biota (Van Eekeren et al., 2008, 2009a,b) have recently been assessed. The general objective of this study is to investigate to what extent biotic soil parameters have indicative and explanatory value in soil quality assessment of grassland on sandy soils. At one sampling point, abiotic and biotic soil parameters were measured together with process parameters underlying ecosystem services in 20 production grasslands with comparable management histories. In the growing season, grass yield at 0 kg N ha^{-1} and response of grass yield to N fertilizer was measured in experimental plots fertilized with 0, 150 and 300 kg N ha^{-1} yr^{-1}. Measured soil parameters were used to explain process parameters underlying the following ecosystem services:

1.1. Soil structure maintenance

We hypothesized that soil structure maintenance is positively influenced by SOM, and the biomass of roots, bacteria, fungi and earthworms.

1.2. Water regulation

We hypothesized that water regulation is positively correlated with the biomass of earthworms, thanks to the positive effect of their burrowing activities on soil structure, penetration resistance and hence water infiltration and root development.

1.3. Nutrient supply

We hypothesized that nutrient supply of the soil is positively influenced by the quality of SOM (organic C, total N and C/N ratio) and correlated with the abundance and biomass of micro-organisms, microbivorous grazers (protozoans, nematodes, micro-arthropods), and their predators (nematodes and mites).

1.4. Grass production

We hypothesized that the dry matter yield of grassland and the response to N fertilization are explained by one or more of the process parameters for nutrient supply.

2. Materials and methods

2.1. Experimental sites

The experiment was conducted in 2006, on 20 permanent grasslands on sandy soil distributed over ten conventional dairy farms. The grasslands were selected using the following criteria: sandy soil, minimum age of the sward of three years, and a botanical composition with a minimum of 65% grass cover (mainly *Lolium perenne* L.) and maximum 2% legumes (Table 1). On 15 grasslands, the historical management was mixed grazing and cutting, while on five it was purely cutting. On average, the 20 grasslands received, the year before the experiment was conducted, 222 ± 70 kg N ha^{-1} in inorganic fertilizer and 260 ± 67 kg N-total - ha^{-1} in organic fertilizer, mainly cattle manure slurry. On all grasslands, the manure slurry was slit injected.

On each grassland, an experimental field (15 m × 9 m) was laid out in February 2006. The first 10 m of the experimental field was split into three plots of 10 m × 3 m and the last 5 m in one plot of 5 m × 9 m. Over the three plots (10 m × 3 m), a fertilization treatment was randomized. Plots were fertilized with 0, 150 and 300 kg N ha^{-1} yr^{-1} with calcium ammonium nitrate (CAN, 27% N), respectively. Of the annual N fertilization, 33% was given before the first growing period, 27% before the second, 23% before the third and 17% before the fourth and last growing period. The remaining 5 m × 9 m plot was not fertilized with N and was used to determine soil quality properties. All plots, except the 5 m × 9 m plot, received ample fertilization of P, K and S.

Weather data were recorded at the weather stations in Heino and IJsselstein. Average rainfall during the growing season 2006 (1 May until 31 October) was 570 mm (Heino) and 521 mm (IJsselstein). The average temperature during this period was 14.0 °C

Table 1
Coordinates, soil type, age and botanical composition of the 20 grasslands sampled.

No.	Coordinates		Soil type (USDA)	Age (years)	Botanical composition (%)			
	'North	'East			*L. perenne*	Other grasses	Legumes	Herbs
1	52°44	6°27	Plaggeptic Haploquod	8	64	28	0	8
2	52°44	6°27	Plaggeptic Haploquod	3	73	27	0	0
3	52°54	6°27	Typic Haploquod	6	68	11	0	21
4	52°54	6°27	Typic Haploquod	6	75	9	0	17
5	52°42	6°21	Plaggeptic Haploquod	7	85	11	0	4
6	52°42	6°21	Typic Haploquod	7	82	12	0	6
7	52°49	6°31	Typic Haploquod	4	76	17	2	6
8	52°50	6°29	Typic Humaquept	6	69	24	0	7
9	52°40	6°22	Typic Humaquept	8	63	35	0	3
10	52°41	6°22	Plaggeptic Haploquod	5	88	9	0	4
11	51°51	5°88	Typic Haploquod	4	50	32	1	18
12	51°51	5°89	Typic Haploquod	4	66	25	0	10
13	51°54	5°84	Typic Haploquod	8	80	9	0	11
14	51°54	5°84	Typic Haploquod	12	17	72	0	11
15	51°53	5°90	Typic Haploquod	4	94	2	0	4
16	51°53	5°90	Typic Haploquod	4	95	3	1	2
17	51°54	5°84	Typic Haploquod	5	92	6	0	2
18	51°50	5°84	Typic Haploquod	5	95	5	0	1
19	51°58	5°83	Plaggeptic Haploquod	4	95	1	0	4
20	51°58	5°83	Typic Haploquod	4	97	2	0	2

(Heino) and 14.5 °C (IJsselstein). The summer season of 2006 was characterized by a very dry month of July.

2.2. Soil sampling

Soil samples were taken between 28 April and 2 May 2006. Per unfertilized 5 m × 9 m plot, a field-moist bulk sample of 70 cores (0–10 cm, ∅ 2.3 cm) was collected randomly, sieved through 1 cm mesh, homogenized and stored at 4 °C until analysis. The bulk sample was split into sub-samples for abiotic and biotic (nematode and microbiological) analysis. An overview of all measurements is given in Table 2. Details of sampling methods are given in the following sections.

2.3. Abiotic soil parameters

Soil moisture contents were measured in the 5–10 cm layer below the soil surface, in three undisturbed ring samples containing 100 cm^{-3} soil per unfertilized 5 m × 9 m plot. Samples were weighed, oven-dried (70 °C), and re-weighed to determine moisture content.

Soil dry matter content was determined after oven-drying of approximately 30 g of the bulk sample (in duplicate) at 105 °C. Prior to chemical analysis, samples were oven-dried at 40 °C. Soil acidity of the oven-dried samples was measured in 1 M KCl (pH-KCl). Soil organic matter (SOM) was determined by loss-on-ignition (Ball, 1964). Total Carbon (C) was measured by incineration of dry material at 1150 °C, after which the CO_2 produced was determined by an infra-red detector (LECO Corporation, St. Joseph, Mich., USA). Hot Water-extractable Carbon (HWC) was analyzed according to the method of Ghani et al. (2003). Field-moist samples were extracted with 30 ml distilled water for 30 min centrifuged for 20 min and filtered. Then a further 30 ml distilled water was added to the sediments, shaken for 10 s and left for 16 h in a hot-water bath at 80 °C. Dissolved Organic Carbon (DOC) was determined by extraction in $CaCl_2$. For determination of total Nitrogen (N), evolved gasses after incineration were reduced to N_2 and detected with a thermal-conductivity detector (LECO Corporation, St. Joseph, Mich., USA). Several phosphorous fractions (aluminum-bound, water-extractable and total P) were determined according to standard methods (Bronswijk et al., 2003). Total potassium (K) in solution was determined using flame photometry after extraction of soil with HCl (0.1 M) and oxalic acid (0.5 M) in a 1:10 M:V ratio and filtration (Bronswijk et al., 2003).

Soil particle analysis was done by a Beckman Coulter LS-230 laser with software version 3.01 and firmware version 2.02. Particle analysis was performed after removal of $CaCO_3$ with 1 M HCl (at 80–95 °C), and addition of de-ionized water, and of 30% H_2O_2 to remove organic matter (at 80–95 °C).

Table 2
Overview of soil parameters measured.

Set	Type of parameter	Parameter
Abiotic (17)	Physical (6)	Clay (<2 μm), silt (>2<50), loam (<50), fine sand (>50<210), coarse sand (>210<2000), Soil moisture
	Organic matter and its characteristics (7)	SOM, HWC, DOC, C-total, Total N, C/N ratio, C-percentage of SOM
	Inorganic chemical (4)	pH-KCl, P-total, P-Al, K-HCl
Biotic (39)	Roots (1)	Root biomass
	Earthworms (7)	Total number, total biomass, number of taxa, number and percentage of epigeic and endogeic adults
	Enchytraeids (9)	Total number, total biomass, number of taxa, number and percentage of *Fridericia*-group, *Marionina*-group and *Enchytraeus*-group
	Micro-arthropods (5)	Total number of micro-arthropods, number and percentage of mites, number and percentage of Collembolan
	Nematodes (11)	Total number of nematodes, number of taxa, Maturity Index, number and percentage of fungivorous nematodes, of herbivorous, bacterivorous, and predacious nematodes
	Microbial (6)	Bacterial biomass, bacterial activity, fungal biomass, fungal activity, physiological activity and diversity of bacterial community
Process (17)	Soil structure maintenance (8)	Bulk density, penetration resistance at 0–10 cm, 10–20 cm, 20–30 cm and 30–40 cm, percentage of crumbs, sub-angular blocky elements, and angular blocky elements
	Water regulation (5)	Water infiltration rate, number of earthworm burrows at 10 cm and 20 cm depth, number of roots at 10 cm and 20 cm depth
	Supply of nutrients (4)	Potential C mineralization, potentially mineralizable C, potential N mineralization, potentially mineralizable N

(..) = Number of parameters.

2.4. Biotic soil parameters

2.4.1. Roots

Three soil cores (0–10 cm, ∅ of 8.0 cm) per unfertilized 5 m × 9 m plot were taken randomly to determine the root biomass. The soil in the samples was thoroughly washed out with water over a sieve with a mesh size of 2 mm. All roots were collected, oven-dried at 70 °C and the dry matter of the roots was measured.

2.4.2. Earthworms

Earthworms were sampled in 2 blocks (20 cm × 20 cm × 20 cm) per unfertilized 5 m × 9 m plot. The blocks were transferred to the laboratory where the whole block was broken down and the earthworms were hand-sorted, counted, weighed and fixed in alcohol prior to identification. Numbers and biomass were expressed per m^2. Adults were identified according to species. A distinction was made between (1) epigeic species (pigmented, living superficially in the litter layer, little burrowing activity), (2) endogeic species (living in burrows at approximately 10–15 cm depth) and (3) anecic species (relatively large worms, living in vertical burrows from which they collect dead organic matter from the surface at night) (Bouché, 1977). The sampling method with blocks of 20 cm × 20 cm × 20 cm is known to underestimate especially the number of *L. terrestris*.

2.4.3. Enchytraeids

Per unfertilized 5 m × 9 m plot, three enchytraeid samples were taken using a separable core sampler of 15 cm length with a diameter of 5.8 cm, holding 6 PVC rings of 2.5 cm high. The enchytraeids were extracted from the soil in the rings with a modified wet extraction method (Didden and Römbke, 2001; Römbke et al., 2006). The organisms were counted, measured and identified using a light microscope. Adults were identified according to species and juveniles to genus. Based on length, the fresh weight was calculated according to Abrahamsen (1973). The observed species were subdivided into three functional groups (1) *Fridericia*, (2) *Marionina* and (3) *Enchytraeus* (Didden and Römbke, 2001).

2.4.4. Micro-arthropods

Per unfertilized 5 m × 9 m plot, three samples for micro-arthropods were collected with a core sampler of 15 cm length with a diameter of 5.8 cm, holding 3 PVC rings of 2.5 cm high. Micro-arthropods were extracted from the soil by placing the soil sample rings in a Tullgren funnel (Siepel and van de Bund, 1988; Römbke et al., 2006). The temperature in the upper part of the funnel was set at 30 °C and kept at 5 °C in the lower part. The organisms moved downwards to escape the heat, dropped through a funnel and collected in a bottle containing 70% ethanol. The total extraction time was one week. Collembola and mites were counted separately but not identified.

2.4.5. Nematodes

For determination of number and species of nematodes, a subsample of 450 g of field-moist soil was taken from the bulk sample. Approximately 100 g of this was put in a suspension from which the free-living nematodes were extracted, 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 according to genus and, whenever possible, to species. The Maturity Index was calculated as the weighted mean of the individual cp-values, in accordance with Bongers (1990) and Bongers et al. (1995) as an index of soil quality.

2.4.6. Microbial parameters

Microbiological analyses were performed on another 200 g of field-moist soil, adjusted to 50% of the water-holding capacity, and pre-incubated at 12 °C for four weeks. Incubation was performed to stabilize soil conditions (Bloem et al., 2006). After pre-incubation, fungal and bacterial biomass, bacterial growth rate and Community-Level Physiological Profiles (CLPP) were determined. 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 the bacterial cell volume. Bacterial growth rate was determined as the incorporation of [^{3}H]thymidine 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).

The CLPPs of the bacterial communities in the soil extracts were determined with ECO-plates from BIOLOG Inc. (Hayward, USA). These plates contain a triplicate set of 31 different carbon substrates, a control, a freeze-dried mineral medium and a tetrazolium redox dye. For each bacterial extract, a dilution series was made using 10 mM BisTris buffer at pH 7. Each dilution series (3^{-1} until 3^{-12}) was used to inoculate four ECO-plates with a volume of 100 µl per well. The color formation in the plate was measured every 8 h for 7 days with a plate reader spectrophotometer at 590 nm. The CLPPs were calculated from the color formation in the wells, and corrected for inoculum density using a regression approach applied to the average well color development (AWCD) as described by Rutgers et al. (2006). To survey the bacterial community activity in the ECO-plate, the AWCD was calculated after 7 days of incubation. The CLPP-slope parameter was calculated from the color development in the ECO-plates. This parameter indicates the rate at which the capacity of the soil to degrade a set of carbon and energy substrates disappears upon dilution. A low slope parameter is indicative of a slow disappearance rate and can be considered as a measure of high physiological diversity (Rutgers et al., 2006). In addition, the amount of extracted soil necessary for conversion of 50% of all substrates in ECO-plates was determined, which is a measure of physiological activity in the bacterial community. A low amount of extracted soil necessary is indicative of a high degree of activity.

2.5. Soil process parameters

2.5.1. Soil structure maintenance

Soil bulk density was measured in the 5–10 cm layer below the soil surface, in three undisturbed ring samples containing 100 cm^{-3} soil per unfertilized 5 m × 9 m plot. Rings were weighed, oven-dried (70 °C), and re-weighed. Penetration resistance was measured using an electronic penetrometer (Eijkelkamp, Giesbeek, The Netherlands) with a cone diameter of 1 cm^2 and a 60° apex angle. Cone resistance was recorded per cm of soil depth and expressed as an average value of 20 penetrations per unfertilized 5 m × 9 m plot in the soil layers of 0–10 cm, 10–20 cm, 20–30 cm and 30–40 cm. Soil structure was determined in 2 blocks (20 cm × 20 cm × 10 cm) per unfertilized 5 m × 9 m plot. Soil in this block was assigned by visual observation of crumbs, sub-angular blocky elements and angular blocky elements (FAO, 2006). These were weighed and expressed as a percentage of total fresh soil weight. On horizontal surfaces (20 cm × 20 cm) at 10 cm and 20 cm depth, the total number of roots was counted and expressed per m^2.

2.5.2. Water regulation

Per unfertilized 5 m × 9 m plot, water infiltration was measured at 3 randomly chosen spots. A PVC pipe of 15 cm high (∅ of 15 cm) was driven into the soil to a depth of 10 cm, after which 500 ml of water was poured into the ring. The number of minutes it took for the 500 ml water to infiltrate was recorded and calculated to give an infiltration rate per minute. Earthworm burrows with a diameter >2 mm were counted on horizontal surfaces (20 cm × 20 cm) exposed at 10 cm and 20 cm depth. On the same horizontal surfaces, the number of roots was counted.

2.5.3. Supply of nutrients

The potential C mineralization was measured in soil which was homogenized, sieved (5 mm mesh size) and brought to 50–60% of

the water-holding capacity. Sub-samples of 200 g soil were incubated in the dark at 20 °C in 1.5 l air-tight jars supplied with a gas septum. CO_2 evolution was measured weekly by gas chromatography over a 6-week period of incubation. The gas chromatograph was a Carlo Erba 6000 with a column switching system, equipped with a 4 m Porapak q and a 2 mmol sieve 5A column. The detector (HWD) temperature was 180 °C, the column temperature was 50 °C, and the injection volume was 1 ml (Bloem et al., 1994). Potential C mineralization was expressed as mg C respired kg^{-1} soil $week^{-1}$, averaged over the last five weeks. The first week's results were not used in order to avoid soil homogenization effects.

Potentially mineralizable C was measured by incubation of dried (35 °C for 48 h) and re-wet soil samples for seven days at 20 °C and 50% WHC. During this period, CO_2 was absorbed in alkali (1 N KOH) followed by titration with 0.1 N HCl (Pell et al., 2006).

The potential N mineralization rate (aerobic) was determined in the same jars used for potential C mineralization measurements, and during the same 6-week incubation period. Potential N mineralization was determined as the increase in mineral N (ammonium plus nitrate) in the five last weeks. The first week's results were not used in order to avoid soil homogenization effects. NH_4 and NO_3 contents were determined by Segmented Flow Analysis, after extraction of 80 g of field-moist soil with 200 ml of 1 M KCl, shaken for 1 h and filtered over a paper filter.

The potentially mineralizable N (anaerobic) was determined by anaerobic incubation of a soil sample under water 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_4^+ to NO_3^- (nitrification) and uncontrolled N losses by denitrification cannot occur.

2.6. Grass production

Grass was harvested four times (10–15 May, 26 June, 11–15 August and 2–3 October). Plots were cut at a stubble height of 6 cm, using a 'Haldrup' small-plot harvester (J. Haldrup a/s, Løgstør, Denmark). Grass was weighed and sampled for dry matter (DM) and total N analysis. DM was determined after drying at 70 °C. Dry material was analyzed for total N (Kjeldahl) and residual moisture content (105 °C).

Dry Matter (DM) yield as a function of N level was modeled based on an exponential curve:

$$Y = \alpha_0 i + \left(\alpha_1 i \times \left(1 - e^{-\rho * Ngift}\right)\right) + \varepsilon_{ij}$$

with the terms:

Y, DM yield;
$\alpha_0 i$, DM yield intercept, or DM yield of field i with 0 kg N ha^{-1};
$\alpha_1 i$, DM yield response to N fertilizer when N level is infinite; so with infinite N level, maximal yield per field is $\alpha_0 + \alpha_1 i$;
ρ, velocity parameter for yield increase;
ε_{ij}, random variance between plots within a field , $\varepsilon_{ij} \sim N(0, \sigma_V^2)$.

The model has been adapted in Genstat 8 with Residual Maximum Likelihood (REML). The non-linear parameters have been estimated according to an iterative procedure, based on 1st order Taylor-approach.

N yield as function of N level was modeled by a linear trend:

$$Y = \beta_{0i} + \beta_{1i} \times Ngift + \varepsilon_{ij}$$

with the terms:

Y, N yield;
β_{0i}, N yield intercept, or N yield of field i with 0 kg N ha^{-1};
$\beta_1 i$, N yield response to N fertilizer; or the slope of the linear correlation between N yield and N application;
ε_{ij}, random field effect, $\varepsilon_{ij} \sim N(0, \sigma_V^2)$.

Assumptions were that the variance on each field was the same and that the correlation between N yield and N application was linear.

In the statistical analyses of the experiment, we used the intercepts of these two equations representing the DM yield and N yield at 0 kg N ha^{-1}. Furthermore, we used the maximum DM yield and slope of the N yield representing the response to N fertilization or the 'apparent' N recovery of fertilizer (ANR).

The nitrogen supply capacity of the soil, defined as the non-fertilizer N supply including atmospheric deposition (Hassink, 1995a), was calculated from soil total N, according to the Dutch grassland fertilization recommendation. For a grassland with an age of 4–6 years, the formula used was $78 + 28.36 \times (\text{g total N/kg soil})^{1.0046}$(http://www.bemestingsadvies.nl).

2.7. Statistical analyses

Data analysis was performed with Matlab (version 7.6.0 R2008a, Mathworks). If necessary, parameters were log-transformed for heteroscedacity to improve the normality of their distributions. Pearson correlations were calculated for all pairs of parameters. Cross-validated stepwise regression was applied to find subsets of parameters that most accurately explained the response parameters. Response parameters were either soil process parameters underlying ecosystem services (section 2.6) or grass production parameters (section 2.2). Soil process parameters were explained by a set of soil parameters (abiotic or biotic) or a combination of the two. Grass production parameters were explained by a set of abiotic or biotic soil or process parameters or all possible combinations of these. Sets of potential regression models were generated using a minimum of one and a maximum of three variables. For each maximum number of parameters, the best regression model was selected by the highest cross-validated R^2. Final selection of reliable subsets of explanatory parameters was based on random permutation tests. Random permutation tests were performed on the complete stepwise regression procedure described. The permuted response parameter and the true response parameter were restricted to have a Pearson correlation below 0.5. For each maximum number of parameters, we tested in 999 permutations whether the permuted cross-validated R^2 was equal to or above 90% of the true cross-validated R^2. In other words, we assessed whether there was a significant 10% gap between the true cross-validated R^2 and the distribution of all permuted cross-validated R^2s. Random permutation tests resulting in a P-value below 0.05 were considered valid.

3. Results

This section starts with the analysis of soil processes underlying the ecosystem services soil structure maintenance, water regulation and nutrient supply. They are related to abiotic and/or biotic soil parameters. This is followed by the explanation of grass production by abiotic or biotic soil or process parameters or a combination of these. In general, data examination followed the pattern of correlation analysis followed by a stepwise regression procedure for the different process parameters underlying an ecosystem service. Many significant correlations were established (Table 3, Table 7, Annex 1).

Table 3
Highest 3 correlations of soil process parameters underlying the different ecosystem services and abiotic and biotic soil parameters. Complete overview of correlations in Annex 1.

Service and parameters	Highest correlating abiotic and biotic parameters (all significant at the <0.05 probability level)
Soil structure maintenance	
Bulk density	HWC (−0.64), SOM (−0.56), Number of bacterivorous nematodes (−0.55)
Penetration resistance 0–10 cm	Number of earthworm taxa (−0.63), Number of earthworms (−0.62), Number of endogeic earthworms (−0.55)
Penetration resistance 10–20 cm	Fungal activity (+0.78), Number and % of *Enchytraeus* enchytraeids (−0.65, −0.61)
Penetration resistance 20–30 cm	Number of herbivorous nematodes (−0.62), Number of *Enchytraeus* enchytraeids (−0.6), total N (−0.57
Penetration resistance 30–40 cm	Number of epigeic earthworms (+0.61), Number of enchytraeids (−0.56), % Collembola (−0.56)
% Crumbs	Number of earthworms (+0.51)
% Sub-angular blocky elements	Organic C (−0.54), SOM (−0.54), Number of earthworm taxa (0.47)
% Angular blocky elements	Number of earthworms (−0.46), Number of earthworm taxa (−0.46)
Water regulation	
Water infiltration rate	Fungal activity (+0.6), Number and % of *Enchytraeus* enchytraeids (−0.54, −0.50)
Number of earthworm burrows at 10 cm	Bacterial activity (−0.51), Number of mites (+0.52), Bacterial biomass (+0.6)
Number of earthworm burrows at 20 cm	Number of nematode taxa (+0.69), Number and % of *Marionina* enchytraeids (−0.66, −0.64)
Number of roots at 10 cm	Number of bacterivorous nematodes (−0.56), %.*Enchytraeus* enchytraeids (−0.55). Number of *Marionina* enchytraeids (+0.47)
Number of roots at 20 cm	Number of micro-arthropods (−0.61), Physiological activity (+0.47), Number of Collembola (−0.46)
Supply of nutrients	
Potentially mineralizable C	Number of nematode taxa (+0.73), Total N (+0.71), % *Marionina* enchytraeids (−0.63)
Potentially mineralizable N	Total N (+0.83), Number of nematode taxa (+0.76), % *Marionina* enchytraeids (−0.75)
Potential C mineralization	%Mites (+0.55), Silt (+0.52), Loam (+0.52)
Potential N mineralization	%Collembola (+0.54), Number of enchytraeids taxa (+0.53), Coarse sand (−0.53)

3.1. Soil structure maintenance

The process parameters measured to assess the service of soil structure maintenance were bulk density, penetration resistance and visual soil structure (Table 2). The results are examined in this sequence.

Bulk density had the highest correlation with the abiotic soil parameters Hot Water-extractable Carbon and SOM (Table 3, Annex 1). In the stepwise regression procedure, a significant model could only be established using HWC (Table 4).

Penetration resistance is a result of soil density, grinding action and humidity of the soil. In the 0–10 cm soil layer it was negatively correlated with biotic soil parameters such as number of earthworm taxa, total number of earthworms and number of endogeic adult earthworms (Table 3, Annex 1). However, these correlations did not lead to a significant model after the random permutation tests on the complete stepwise regression procedure with one or more parameters. Penetration resistance at 10–20 cm was positively correlated with fungal activity, and negatively with the number and percentage of *Enchytraeus* enchytraeids (Table 3, Annex 1). In contrast to the penetration resistance at 0–10 cm, the resistance at 10–20 cm could be explained by several regression models with biotic soil parameters in which fungal activity was the main explanatory parameter (Table 4). Penetration resistance at 20–30 cm and 30–40 cm were amongst others negatively correlated to the total number of enchytraeids (Table 3, Annex 1), but none of the models was significant after the random permutation tests.

For the percentage of soil crumbs and angular blocky elements, no regression models could be fitted. Nevertheless, the percentage of crumbs showed a positive correlation with the total number of earthworms and a negative correlation with penetration resistance at 0–10 cm (Table 3, Annex 1). Since soil crumbs and angular blocky elements are the other's antithesis, this was vice versa for angular blocky elements. The percentage of sub-angular blocky elements was negatively correlated with SOM and parameters characterizing organic matter quality. The percentage of sub-angular blocky elements could be significantly explained by a model containing SOM and soil C/N ratio as parameters (Table 4).

Table 4
Explanation of soil process parameters underlying the ecosystem service soil structure maintenance. The cross-validated R^2 and *P*-values of all significant ($P < 0.05$) models were calculated using only one parameter of either the set of abiotic (A) or biotic (B) soil parameters, or a combination of the two. The same procedure was followed for a maximum of two or three parameters.

Response parameter	cvR^2	*P*-value	Set	Explanatory parameter(s)
Bulk density	0.27	0.039	A	−HWC
Penetration resistance				
0–10 cm	–	NS	–	
10–20 cm	0.51	0.003	B	+Fungal activity
	0.69	0.005	B	+Fungal activity, −Number of *Enchytraeus* enchytraeids
	0.73	0.016	B	+Fungal activity, −Number of *Enchytraeus* enchytraeids, +Number of enchytraeid taxa
20–30 cm	–	NS	–	
30–40 cm	–	NS	–	
Soil structure elements				
Crumbs %	–	NS	–	
Sub-angular %	0.44	0.034	A	−SOM, −C/N ratio
Angular %	–	NS	–	

3.2. Water regulation

Water regulation was assessed using the process parameters water infiltration rate per minute, the number of earthworm burrows and the number of grass roots at 10 and 20 cm depth.

From the abiotic and biotic soil parameters, water infiltration rate was, like penetration resistance at 10–20 cm, correlated with fungal activity, and the number and percentage of *Enchytraeus* enchytraeids (Table 3, Annex 1). However, the water infiltration rate had a stronger negative correlation with penetration resistance at 10–20 cm and 20–30 cm (Annex 1). In the stepwise regression procedure, the water infiltration rate could only be explained by penetration resistance at 10–20 cm when one parameter out of 16 soil process parameters was allowed (Table 5).

The number of earthworm burrows at 10 and 20 cm depth was not significantly correlated with water infiltration. Earthworm burrows at 10 cm depth were correlated with abiotic and biotic soil parameters (Table 3, Annex 1). However, the number of earthworm

Table 5
Explanation of soil process parameters underlying the ecosystem service water regulation. The cross-validated R^2 and *P*-values of all significant ($P < 0.05$) models were calculated using only one parameter of either the set of abiotic (A) or biotic (B) soil parameters, or a combination of the two. The same procedure was followed for a maximum of two or three parameters. For infiltration rate also a model with 16 soil process (P) parameters was tested.

Response parameter	cvR^2	*P*-value	Set	Explanatory parameter(s)
Water infiltration rate	0.36	0.016	P	−Penetration resistance at 10–20 cm
Earthworm burrows				
10 cm depth	–	NS	–	
20 cm depth	0.58	0.011	A,B	+DOC, −Bacterial activity
	0.70	0.025	A,B	+DOC, −Bacterial activity, +P-total
Number of roots				
10 cm depth	0.66	0.049	B	+Total biomass of enchytraeids, −Number of bacterivorous nematodes, −Number of *Enchytraeus* enchytraeids
20 cm depth	–	NS	–	

Table 6
Explanation of soil process parameters underlying the ecosystem service supply of nutrients. The cross-validated R^2 and *P*-values of all significant ($P < 0.05$) models were calculated using only one parameter of either the set of abiotic (A) or biotic (B) soil parameters, or a combination of the two. The same procedure was followed for a maximum of two or three parameters.

Response parameter	cvR^2	*P*-value	Set	Explanatory parameter(s)
Field respiration	–	NS	–	
Potential C mineralization	–	NS	–	
Potential N mineralization	–	NS	–	
Potentially	0.36	0.016	A	+Total N
mineralizable C	0.41	0.019	B	+Number of nematode taxa
Potentially	0.61	0.001	A	+Total N
Mineralizable N	0.51	0.011	B	+Number of nematode taxa
	0.72	0.002	A	+Total N, −Loam fraction
	0.65	0.016	B	−Percentage *Marionina* enchytraeids, +Percentage Collembola within micro-arthropods
	0.71	0.005	A,B	+Total N, −Bacterial activity
	0.79	0.013	A,B	+Total N, +Number of nematode taxa, −Number of adult endogeic earthworms

burrows at 10 cm depth could not be explained with a significant model with one or more abiotic or biotic soil parameters. The number of earthworm burrows at 20 cm depth was correlated with several abiotic and biotic soil parameters. In the stepwise regression procedure, the number burrows was explained by DOC, bacterial activity and soil total P when a maximum of three parameters was allowed out of the combined set of 17 abiotic and 39 biotic parameters (Table 5).

The number of roots at 10 cm depth was negatively correlated with number of bacterivorous nematodes and percentage of *Enchytraeus* enchytraeids, and positively with soil bulk density (Table 3, Annex 1). In the stepwise regression procedure, the number of roots was explained by total biomass of enchytraeids, number of bacterivorous nematodes and number of *Enchytraeus* enchytraeids (Table 5). The number of roots at 20 cm was positively correlated with the number of micro-arthropods, but did not result in an explanatory model (Table 3, Annex 1)

3.3. Supply of nutrients

The nutrient supply capacity of the soil depends on the composition of the organic material and the decomposition and mineralization processes. In these processes bacteria, fungi, microbivorous fauna and their predators play an important role. The ecosystem service supply of nutrients was assessed with the process parameters potentially mineralizable C and N, and potential C and N mineralization.

Potentially mineralizable C and N were positively correlated ($r = +0.66$, $P = 0.002$) (Annex 1). Moreover, both parameters were positively correlated with soil total N, and biotic soil parameters that are in turn related to total N. Amongst others, soil total N was positively correlated with total number of nematodes and number of nematode taxa, and negatively with percentage of *Marionina* enchytraeids (Table 3, Annex 1). In regression models, the potentially mineralizable C and N was explained by total N or number of nematode taxa, in case only one abiotic or biotic soil parameter was allowed (Table 6). In a model with two abiotic parameters, total N and loam fraction explained a higher percentage of the variance of the potentially mineralizable N. When all abiotic and biotic soil parameters were allowed in the regression model, the bacterial activity explained extra variation above total N, whereby bacterial activity was again correlated with loam fraction.

Potential C mineralization and potential N mineralization were not significantly correlated with each other, but were correlated with a variable number of contrasting biotic soil parameters (Table 3, Annex 1). The potential N mineralization was positively correlated with the fungal biomass, the number of predaceous nematodes, the Maturity Index, the number of *Fridericia* enchytraeids and the percentage of Collembola, and negatively with the number of mites. The potential C mineralization was negatively correlated with the bacterial biomass, the Maturity Index and the percentage of endogeic earthworms, and positively correlated with the percentage of mites. However, despite several correlations, potential C mineralization and potential N mineralization could not be significantly explained by a regression model with one or more parameters from the abiotic and/or biotic soil parameter sets (Table 6).

3.4. Grass production

3.4.1. DM and N yield intercept

Dry Matter (DM) yield intercept and the Nitrogen (N) yield intercept were used as two standards for grass production parameters at 0 kg N ha^{-1} fertilization. The DM yield intercepts over the different experimental fields ranged from 3363 to 10120 kg DM ha^{-1} (Fig. 1). N yield intercepts ranged from 78 kg to 263 kg N ha^{-1} (Fig. 2).

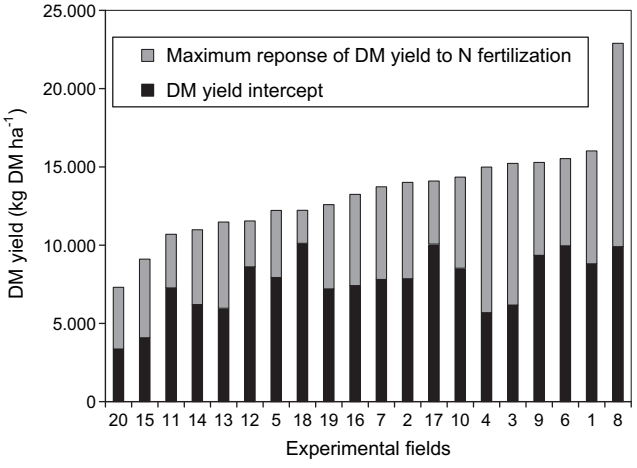

Fig. 1. DM yield intercept and maximum response of DM yield to N fertilization.

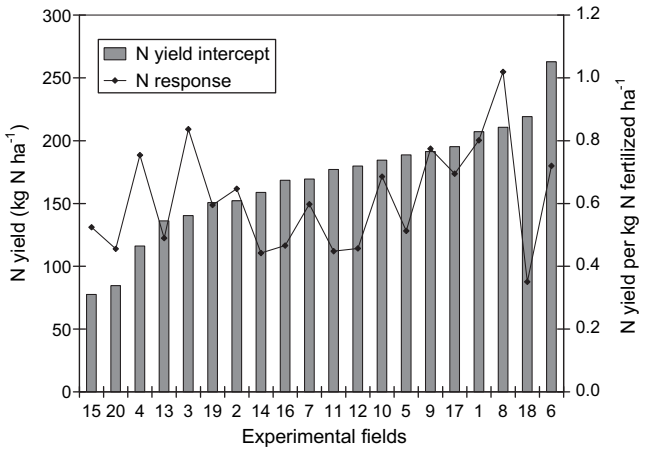

Fig. 2. N yield intercept (kg N ha^{-1}) and response of N yield to N fertilization (N yield per kg N fertilized ha^{-1}).

DM yield intercept was correlated among others with soil moisture, SOM and total biomass of enchytraeids (Table 7). Soil moisture was selected out of 17 abiotic soil parameters in a significant model ($P = 0.002$) with a cross-validated R^2 of 0.67 (Table 8). One percent of soil moisture in the 5–10 cm soil layer in spring explained 427 kg DM yield ha^{-1}. When soil moisture was removed from the abiotic data set, SOM was the next-best parameter ($cvR^2 = 0.51$, $P = 0.007$). Total biomass of enchytraeids was selected as the best explanatory biotic soil parameter. However, biomass of enchytraeids was also positively correlated with soil moisture and SOM (Annex 1). When two abiotic parameters were allowed, a fine sand fraction was added to the model with soil moisture. When two biotic parameters were allowed, the number of bacterivorous nematodes was added to the model with total biomass of enchytraeids.

DM and N yield intercepts were correlated (Table 7). Correlations with soil parameters were comparable for both intercepts, although ranking in the correlations could differ. Both intercepts were correlated with soil moisture and SOM, but soil moisture explained more

Table 7
Significant correlations of intercept and response of DM yield and N yield on N fertilization with soil quality parameters.

Set	Parameters	Intercept		Response on N fertilization	
		DM yield	N yield	DM yield	N yield
Abiotic soil parameters	DM yield intercept	+1**	+0.93**		
	N yield intercept	+0.93**	+1**		
	Response DM yield N fert.			+1**	+0.86**
	Response N yield N fert.			+0.86**	+1**
	Clay	+0.69**	+0.59**		
	Loam	+0.6**	+0.52*		
	Silt	+0.54*	+0.47*		
	SOM	+0.77**	+0.82**		
	C-percentage		−0.53*		
	C-tot	+0.66**	+0.67**		
	C/N ratio			−0.5*	−0.62**
	HWC	+0.58**	+0.67**		
	DOC	+0.61**	+0.49*		
	N-tot	+0.72**	+0.77**		
	Soil moisture	+0.84**	+0.77**		
Biotic soil parameters	Number of epigeic adult earthworms		−0.52*		
	Number of endogeic adult earthworms				+0.45*
	% of epigeic adult earthworms				−0.53*
	% of endogeic adult earthworms			+0.48*	+0.67**
	Number of earthworm taxa				+0.41*
	Total number of enchytraeids	+0.72**	+0.61**		+0.69**
	Total biomass of enchytraeids	+0.78**	+0.7**		+0.59**
	Number of *Enchytraeus* enchytraeids			+0.45*	+0.68**
	Number of *Fridericia* enchytraeids				+0.46*
	% of mites				−0.47*
	Total number of nematodes	+0.5*	+0.61**		
	Number of bacterivorous nematodes		+0.52*		
	Number of fungivorous nematodes	+0.45*	+0.47*		
	% of bacterivorous nematodes			−0.62**	−0.59**
	% of herbivorous nematodes			+0.5*	+0.48*
	Maturity Index of nematodes			+0.53*	+0.56*
	Number of nematode taxa		+0.51*		+0.47*
	Fungal biomass				+0.47*
	Bacterial activity	+0.51*			
	Physiological activity	+0.57**	+0.49*		+0.48*
	Root biomass		−0.46*		
Process soil parameters	Bulk density		−0.46*		
	Sub-angular blocky elements				+0.45**
	Penetration resistance 0–10 cm				−0.56**
	Penetration resistance 20–30 cm		−0.47*		
	Penetration resistance 30–40 cm	+0.53*	−0.58**		
	Earthworm burrows at 20 cm			+0.58**	+0.46*
	Potentially Mineralizable N	+0.5*	+0.64**		+0.49*
	Potentially Mineralizable C	+0.45*	+0.57**		

*Significant at the 0.05 probability level, **Significant at the 0.01 probability level.

Table 8
Explanation of grass production parameters from soil quality parameters. The cross-validated R^2 and *P*-values of all significant ($P < 0.05$) models were calculated using only one explanatory parameter of either the set of abiotic (A) or biotic (B) soil parameters or the set of soil process (*P*) parameters, or a combination of the three. The same procedure was followed for a maximum of two or three parameters.

Response parameter	cvR^2	*P*-value	Set	Explanatory parameter(s)
Intercept				
DM yield	0.67	0.002	A	+Soil moisture
	0.50	0.006	B	+Total biomass of enchytraeids
	0.79	0.001	A	+Soil moisture, −Fine sand fraction
	0.59	0.027	B	+Total biomass of enchytraeids, + Number of bacterivorous nematodes
	0.83	0.001	A	+Soil moisture, −Fine sand fraction, +SOM
	0.85	0.005	A,B	+Soil moisture, −Fine sand fraction, +Physiological activity
	0.83	0.004	A,P	+Soil moisture, −Fine sand fraction, +Field respiration
N yield	0.59	0.001	A	+SOM
	0.35	0.025	B	+Total biomass of enchytraeids
	0.68	0.002	A	+SOM, +Soil moisture
	0.56	0.022	B	+Total biomass of enchytraeids, +Number of bacterivorous nematodes
	0.74	0.002	A,B	+SOM, +Total biomass of enchytraeids
	0.70	0.003	A,P	+SOM, +Percentage sub-angular blocky elements
	0.76	0.002	A	+SOM, +Soil moisture, −C-total
	0.79	0.014	A,B	+SOM, +Total biomass of enchytraeids, −Number of taxa of enchytraeids
N response				
DM yield	–	NS	–	
N yield	0.36	0.047	B	+Total number of enchytraeids
	0.58	0.046	B	+Total number of enchytraeids, +Maturity Index of nematodes
	0.7	0.020	B	+Total number of enchytraeids, +Maturity Index of nematodes, −Number of bacterivorous nematodes

of the variance in the DM yield intercept than SOM, whereas SOM explained more of the variance in the N yield intercept (Table 8). When one abiotic parameter was allowed, SOM was selected as the best explanatory parameter for the N yield intercept (cv$R^2 = 0.59$, $P = 0.001$). One gram of SOM per kg dry soil meant 3.21 kg N yield ha^{-1}, on top of a constant of 15.4 kg N ha^{-1}. When SOM was removed from the abiotic soil parameter set, the N yield intercept could be significantly explained from soil moisture (cv$R^2 = 0.52$, $P = 0.003$) or total N (cv$R^2 = 0.48$, $P = 0.007$). With one biotic parameter in the model, total biomass of enchytraeids was selected. Next to correlations with soil moisture and SOM, total biomass of enchytraeids was positively correlated with soil total N (Annex 1). Models with two or three parameters, produced by best sub-set selection, were dominated by SOM as one of the explanatory parameters. When two biotic soil parameters were allowed, N yield intercept was explained by the total biomass of enchytraeids and the number of bacterivorous nematodes, just as we found with the DM yield intercept.

In order to compare the theoretical nitrogen supply capacity of the soil calculated from the total N according to the Dutch grassland fertilization recommendation (section 2.6) with the N yield intercept from this experiment, both were plotted against SOM (Fig. 3). It can be seen that the nitrogen supply calculated from soil total N underestimated the measured N yield intercept from 32 g SOM kg dry soil^{-1} onwards. In this range of grassland soils, the difference between calculated and measured values ranged from −36 to 103 kg N ha^{-1}. On average, it meant a 24% higher nitrogen supply capacity than calculated according to the Dutch grassland fertilization recommendation. These differences are significantly explained by the model that includes soil moisture in addition to C-percentage in SOM (cv$R^2 = 0.62$, $P = 0.006$). When three parameters were allowed, the differences are explained by a model of soil moisture, C-percentage in SOM and total number of mites (cv$R^2 = 0.76$, $P = 0.033$).

3.4.2. Response of DM and N yield to N fertilization

The maximum DM response to N fertilizer ranged from 2109 to 12992 kg DM ha^{-1} on top of the production without fertilizer (Fig. 1). The response of N yield to N fertilizer ranged from 0.35 to 1.02 kg N yield per kg N ha^{-1} applied (Fig. 2). Yield intercepts and yield responses to N fertilization were not correlated (Table 7).

In contrast to the intercepts, the N fertilizer responses were not significantly correlated with the different abiotic soil parameters, except for a negative correlation with the C/N ratio in the soil (Table 7). Both yield response parameters were correlated with numerous biotic soil parameters such as number of *Enchytraeus* enchytraeids, percentage of endogeic adult earthworms, and percentage of bacterivorous nematodes. Despite these correlations, the response of DM yield to N fertilizer could not significantly be explained by a regression model of abiotic and/or biotic soil parameters. However, the response of N yield to N fertilization was significantly explained by the total number of enchytraeids (cv$R^2 = 0.36$, $P = 0.047$) (Table 8) (Fig. 4). On top of the number of enchytraeids, the Maturity Index and the number of bacterivorous

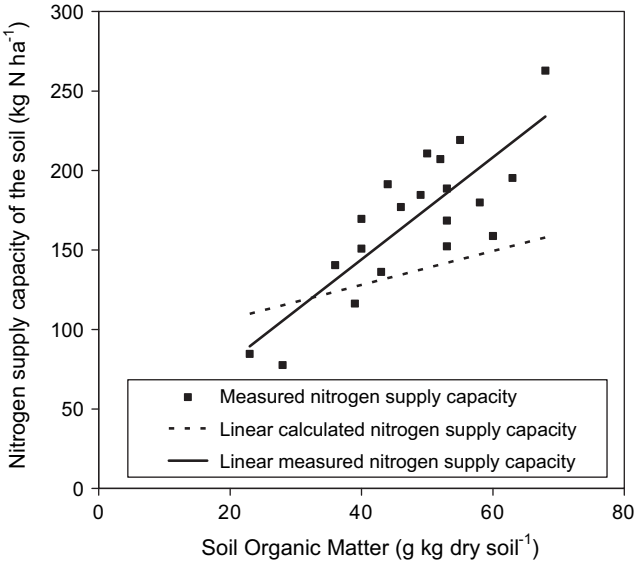

Fig. 3. Measured nitrogen supply capacity (N yield intercept) (kg N ha^{-1}) plotted against SOM (g kg dry soil^{-1}) and its linear regression ($R^2 = 0.67$, cross-validated $R^2 = 0.59$, $P < 0.001$), compared with the nitrogen supply capacity of the soil calculated from total N according to the Dutch grassland fertilization recommendation (section 2.6, http://www.bemestingsadvies.nl).

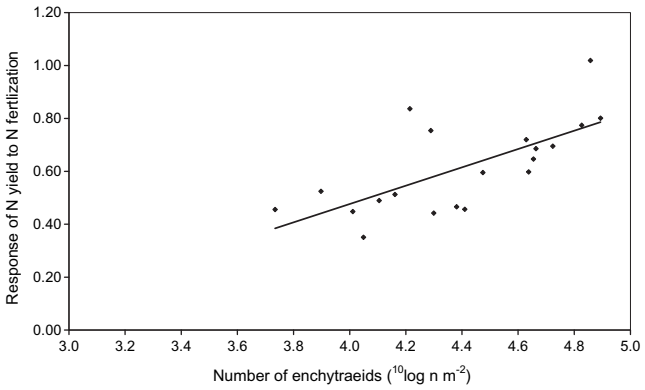

Fig. 4. Linear regression between total number of enchytraeids (^{10}log n m^{-2}) and response of N yield to N fertilization ($R^2 = 0.47$, cross-validated $R^2 = 0.36$, $P = 0.047$).

nematodes explained extra variance in the data set. However, this variance was mainly caused by two grasslands out of the 20. When these two grasslands were left out of the analysis, the cvR^2 increased to 0.53 ($P = 0.006$) with total number of enchytraeids as explanatory parameter. Both yield response parameters had a tendency towards a significant model with three soil process parameters: +Potentially mineralizable N, +Bulk density and −Penetration resistance at 0–10 cm (cv$R^2 = 0.44$, $P = 0.074$ for DM-yield; cv$R^2 = 0.51$, $P = 0.058$ for N yield).

4. Discussion

4.1. Soil structure maintenance

In our experiment, the variation in bulk density was best explained by Hot Water-extractable Carbon (HWC), which is regarded as one of the key labile components of SOM responsible for soil micro-aggregation (Haynes, 2005). Both bulk density and HWC were strongly correlated with SOM. Soil organic particles weigh less than mineral soil particles, which makes soil bulk density highly dependent on SOM (Locher and de Bakker, 1990). Clements et al. (1991) showed that earthworms decreased the soil bulk density. However, the influence of earthworms on bulk density was not confirmed in this study.

Penetration resistance at 0–10 cm could not be explained from the measured abiotic or biotic soil parameters. However, the negative correlations between penetration resistance and biotic soil parameters suggest that earthworms in general and endogeic earthworms in particular, decrease the penetration resistance at 0–10 cm. In experiments by Hoogerkamp et al. (1983) and Clements et al. (1991), earthworms decreased the soil penetration resistance in the 0–10 cm soil layer.

Penetration resistance at 10–20 cm could be explained from the biomass of active fungal hyphae in the 0–10 cm soil layer. This correlation was not expected. Fungal activity was positively correlated with other soil structure parameters that are indicative of a poor soil structure (penetration resistance at 0–10 cm and 20–30 cm, and angular blocky elements) (Annex 1). A poor soil structure may lead to an accumulation of organic material which could have stimulated fungal growth. The positive correlation of fungal activity with surface feeding epigeic earthworms could be an indication of the accumulation of organic material.

Crumbs and angular blocky elements could not be explained from abiotic and/or biotic soil parameters, but they were, like penetration resistance at 0–10 cm, correlated with total number of earthworms. Van Eekeren et al. (2009a) also found a positive correlation between earthworm biomass and the percentage of crumbs in the 0–10 cm soil layer. The percentage of sub-angular blocky elements was explained by SOM and C/N ratio in a regression model, and negatively correlated with C-total and HWC. This suggests that sub-angular blocky elements are indicative of the quality of SOM.

We had hypothesized that the process parameters underlying the ecosystem service soil structure maintenance would be influenced by abiotic soil parameters like SOM and biotic parameters related to the presence and activity of roots, bacteria, fungi and earthworms. We conclude that bulk density and the percentage of sub-angular blocky elements are mainly influenced by SOM quantity and quality. Penetration resistance at 10–20 cm is explained by fungal activity, but the cause and effect are not completely clear. The correlations between penetration resistance at 0–10 cm and percentage of soil crumbs with earthworms suggest a relationship with earthworm activity.

4.2. Water regulation

Effects of biological activity on the service of water regulation have been measured in the field via the number of earthworm burrows, number of roots and via water infiltration. For number of roots and number of earthworms there were some relationships with biotic soil parameters but cause and effect were still difficult to separate. We could not even measure a relationship between earthworm burrows and earthworm biomass, whereas Van Eekeren et al. (2008) established such a relationship in a field experiment.

Water infiltration rate is influenced by soil type, soil texture, soil structure, earthworm burrow numbers, earthworm species, stable organic matter and initial soil water content (Lowery et al., 1996; Sarrantonio et al., 1996; Bouché and Al-Addan, 1997; Edwards and Shipitalo, 1998). In our experiment, water infiltration was not correlated with initial soil water content. The variance in water infiltration was best explained through a regression model with penetration resistance at 10–20 cm as an explanatory parameter. The average penetration resistance in our experiment increased from 1.40 MPa in the 0–10 cm layer to 2.13 MPa in the 10–20 cm layer (data not shown), suggesting that the higher penetration resistance in the 10–20 cm was a barrier to water infiltration.

Our hypothesis of a positive correlation between water infiltration and earthworm activity had to be rejected. A first explanation could be that no anecic species were found in the 0–20 cm soil layer, which may be due to the absence, but more likely the non-capture of *Lumbricus terrestris*. Burrows of these earthworms play an important role in water infiltration (Bouché and Al-Addan, 1997). A second explanation could be that only in experiments with extreme treatments (e.g. with or without earthworms), can an increased water infiltration rate be linked to earthworm presence (Hoogerkamp et al., 1983; Clements et al., 1991) or earthworm burrow numbers (Joschko et al., 1989). A third explanation could be that the negative effect of soil compaction by trafficking of heavy machinery and/or animal trampling precluded a possible positive effect of earthworms on water infiltration in some of the grasslands.

4.3. Supply of nutrients

The correlation between potentially mineralizable N and C has previously been described by Haney et al. (2008). Monaco et al. (2008) measured a correlation between potentially mineralizable N and soil respiration measured in air-dried soil (which we call potentially mineralizable C) in the 0–15 cm and 15–30 cm soil layers. In the current experiment, both parameters were strongly

correlated with total N and number of nematode taxa. The soil parameters, total N and nematode taxa were related. The total number of nematodes increased with a higher soil total N, which probably increased the number of nematode taxa. Few references are available where potentially mineralizable N and C have been related to biotic soil parameters. Some authors have suggested that the potentially mineralizable N measured by anaerobic incubation may serve as a substitute for microbial biomass determinations, since it apparently involves killing and mineralizing the obligate aerobes and correlates with microbial biomass C as determined by fumigation extraction (Drinkwater et al., 1996; Schipper and Sparling, 2000). However, in our experiment, potentially mineralizable N was not correlated with bacterial biomass determined by microscopy (Annex 1). Franzluebbers (1999), Haney et al. (2001) and Hermann and Witter (2002) have suggested that the rapid C mineralization after the drying and re-wetting of samples is indicative for the labile fraction of organic matter, which in turn is closely associated with a soil's agronomic history. Drinkwater et al. (1996) stated that differently managed soils can have similar levels of total N, but very different N mineralization potential, indicating differences in SOM quality. Monaco et al. (2008) confirmed this for potentially mineralizable N when different organic materials were applied, and Van Eekeren et al. (2009b) showed this for potentially mineralizable C when clover was cultivated. In this experiment, the recent management and fertilization history of the different grasslands were rather similar, which could have resulted in a comparable SOM quality, explaining the strong correlation between potentially mineralizable N and total N.

Contrary to potentially mineralizable C and N, potential C and N mineralization were not significantly correlated with total N in the current study. The difference may be explained by the different methods used. Both the potentially mineralizable C and N are biochemical assays, whereby aggregates are destroyed and the total flushes of CO_2 and NH_4^+, respectively, are measured. Potentially mineralizable C was determined after drying and re-wetting of soil which increases the flush of CO_2 in the first week after the disturbance. Potentially mineralizable N was determined by incubation in a slurry at 40 °C under anaerobic conditions. This promotes net N mineralization because less N is immobilized in anaerobic conditions than in aerobic conditions (Patrick and Reddy, 1972; Yadvinder-Singh et al., 2005). In contrast, potential C and N mineralization rates are biological assays measured in moist samples from week 1 to week 6, omitting the first week (flush after disturbance). Different authors (Jenkinson and Powlson, 1976; Franzluebbers et al., 1996; Pell et al., 2006; Canali and Benedetti, 2006) point out that the potential C and N mineralization are representative of the process of mineralization in an undisturbed field-moist soil (basal respiration and basal N mineralization). In our experiment, both potential N and C mineralization were mainly correlated with biotic soil parameters (Annex 1), including microbial biomass, nematode MI and percentage of mites within the micro-arthropods. For these three biotic soil parameters, both potential C and N are the other's antithesis, whereby a high potential N mineralization represents a matured soil food web with predacious nematodes and a high nematode Maturity Index. This in contrast to a high potential C mineralization which has next to a lower nematode Maturity Index, a higher percentage of mites. A higher percentage of mites within the micro-arthropods suggests litter accumulation (Mulder and Elser, 2009; Smeding et al., 2005).

We had hypothesized that nutrient supply of the soil was positively influenced by the quality of SOM and correlated with the abundance and biomass of micro-organisms, microbivorous grazers and their predators. We conclude that potentially mineralizable C and N are mainly determined by soil total N. The potential C and N mineralization are suggested to be more related to biotic soil parameters, whereby the two parameters are the other's antithesis.

4.4. Grass production

4.4.1. DM and N yield intercepts

One of the major objectives of this study was to identify soil parameters that explain grassland production. The DM and N yield intercept of the most productive grassland was three times higher than the least productive grassland. The best abiotic parameter to explain the DM yield intercept was soil moisture, while SOM was the best explanatory parameter for N yield intercept. Hassink (1996) also found a correlation between the N yield at 0 kg N ha^{-1} of mineral soils and SOM in the 0–5 cm soil layer ($r = +0.51$). Hassink (1995a) concentrated his work on the relationship between the N yield at 0 kg N ha^{-1} and the N-organic in the soil and found correlations of $r = +0.38$ in the 0–5 cm layer and $r = +0.70$ in the 0–20 cm layer of sandy and clayey soils. Parfitt et al. (2005) related DM production of grasslands fertilized with 0–90 kg N ha^{-1} to the total N in 0–20 cm ($r = +0.87$) (soils formed in silty mudstones and quartzo-feldspathic loess).

In the Dutch grassland fertilization recommendation (http://www.bemestingsadvies.nl), the nitrogen supply capacity of the soil, defined as the non-fertilizer nitrogen supply including atmospheric deposition, is estimated from soil total N. This relationship is based on the work of Hassink (1995a, 1996). In the range of soils sampled in this experiment, the nitrogen supply capacity on the basis of his work was underestimated in 17 of the 20 grasslands. In this study, part of the difference between the N yield intercept and the nitrogen supply capacity calculated from total N could be explained by soil moisture and C-percentage of the SOM. Apart from these differences, the underestimation of the measured nitrogen supply capacity by the standard calculation method, could be the higher soil C/N ratios in the soils sampled by Hassink (1995a). The latter would be in line with a correction made to the standard calculation method by a commercial laboratory in The Netherlands on the basis of the C/N ratio in the soil (Reijnenveld, personal communication). Differences in C/N ratios in the sandy soils sampled by Hassink (1995a) and this experiment could be explained by the experimental sites and their fertilization history. In the research of Hassink (1995a), some experimental sites were located in the North-East of The Netherlands, which had a relatively high SOM (>100 g kg dry $soil^{-1}$) for sandy soils and probably contained a stable humus with its origin in peat. Moreover, in the experiments of Hassink (1995a), the nitrogen supply capacity was measured on experimental plots without fertilization for several years, while the 0 kg N ha^{-1} plots in the current experiment were without fertilizer for one year only. The fertilization history in our experiment is more representative of day-to-day practice. Therefore, the explanation of the nitrogen supply capacity of grassland on sandy soils with a SOM < 80 g kg dry $soil^{-1}$ could be more realistic with the results of our experiment. The average underestimation of 42 kg N ha^{-1} (31%) for the 17 grasslands with an underestimated nitrogen supply capacity legitimizes new research to modify the currently used recommendations.

DM and N yield intercept could also be significantly explained by total enchytraeid biomass, although the cross-validated R^2 was lower than that of soil moisture and SOM. In the current experiment, the enchytraeid biomass was closely related to soil moisture, SOM or total N in the soil. Mikola et al. (2001) and Van der Wal et al. (2009) measured a positive correlation between enchytraeid abundance and the harvested shoot mass. Since soil organic matter and microbes are the main food sources of enchytraeids (Didden et al., 1994), the observed correlation could be a bottom-up response to food abundance. In our experiment, the number of bacterivorous nematodes explained extra variation of the DM and N yield intercept, in addition

to enchytraeid biomass. These parameters, which were measured in spring before fertilization, are probably indicative of readily available nutrients in the growing season.

Of the process parameters underlying the ecosystem service nutrient supply, potentially mineralizable N was correlated most strongly with both DM and N yield intercept. However, no significant regression model could be established. Parfitt et al. (2005) related potential N mineralization (0–8 weeks) with pasture DM yield ($r = 0.87$; $P < 0.003$) and N yield ($r = 0.95$; $P < 0.001$). The potentially mineralizable N is frequently used as an indicator of the potential N supplying capacity of a soil (Drinkwater et al., 1996; Curtin and McCallum, 2004; Russell et al., 2006).

We had hypothesized that the dry matter yield of grassland would be explained by one or more of the process parameters for nutrient supply. In our experiment, however, an abiotic soil parameter (SOM) best explained the nitrogen supply capacity, and not a soil process parameter such as potentially mineralizable N. Therefore, we conclude that for the explanation of the nitrogen supply capacity of the soil, the focus should be on abiotic parameters such as moisture content, SOM and total N.

4.4.2. Response of DM and N yield to N fertilization

The response of N yield to N fertilization or the 'apparent' N recovery (ANR) of fertilizer by grassland is usually between 50 and 80% (Whitehead, 1995). In the current experiment, the response of N yield to N fertilization ranged from 35 to 102%. For the grassland with the lowest N response, this would mean that 149 kg N ha^{-1} of the average 222 kg N ha^{-1} inorganic fertilizer applied on these 20 grasslands in 2005, was not harvested as N in the grass compared with the grassland with the highest response. This shows the importance of explaining this response in order to better target the N fertilizer, not only from an economic point of view but also to prevent losses to the environment.

The response of DM and N yield to N fertilization could not be explained by abiotic soil parameters, although both responses were negatively correlated with soil C/N ratio (in the range of 11.2–19.1). This appears obvious: with decreasing C/N ratio, less fertilizer N will be immobilized, i.e. more will be available for plant uptake and dry matter production.

The response of N yield to N fertilization could be explained by the total number of enchytraeids. It is remarkable that a biotic parameter explained the response to inorganic N fertilizer. A possible explanation could be that enchytraeids indicate a nutrient-rich environment. However, different from the N yield intercept, the response of N yield to N fertilization was not correlated with other abiotic soil parameters representing a nutrient-rich environment, such as total N. Furthermore, just as by Hassink (1995b), the response of N yield to N fertilization was not related to the N yield intercept (Fig. 3, Table 6). A second explanation could be the effect of the historical grass production on the enchytraeid abundance measured in the spring of 2006. In fact, the enchytraeid abundance could be an indication of the potential production capacity with N fertilization. As already mentioned, Mikola et al. (2001) and Van der Wal et al. (2009) measured a positive correlation between enchytraeid abundance and the harvested shoot mass. Yeates (1968) also postulated that enchytraeids appear to act as an indicator of favorable conditions. A third possible explanation is the role of enchytraeids in decomposition processes. Research of Didden (1993) and Mulder (2006) on clayey as well as sandy soils suggests that enchytraeids are a key functional group of the decomposition community. Enchytraeids have been shown to strongly regulate ecosystem processes of organic matter decomposition and nutrient (C and N) mineralization (Cole et al., 2002; Mulder and Elser, 2009). Therefore, the total number of enchytraeids may be indicative of a balanced decomposition of organic matter and a high N response to N fertilization. We recommend that the mechanisms behind this relationship be further investigated.

The response of N yield to N fertilizer was correlated with potentially mineralizable N and there was a tendency towards an explanatory model with three soil process parameters, including potentially mineralizable N. We had hypothesized that the response to N fertilization would be explained by one or more of the process parameters for nutrient supply. However, we have to conclude that a biotic soil parameter explained more of the variance.

4.5. Biotic soil parameters in soil quality assessment?

The main objective of this study was to investigate to what extent biotic soil parameters have added value in soil quality assessment of grassland on sandy soils. For the explanation of soil process parameters underlying soil ecosystem services, various biotic soil parameters indeed played a role. However, the majority of the process parameters and the N yield intercept were best explained by "traditional" abiotic parameters such as soil moisture content, SOM and total N. Only for the explanation of the N response to N fertilization, a biotic parameter (number of enchytraeids) had added value over and above abiotic parameters. An explanation for this could be that the fertilization on these 20 grasslands was so high in the past that ecosystem self-regulating processes were overruled. Ritz et al. (2009) stated that the majority of soil processes are driven by soil biota. This would suggest that abiotic parameters are intermediate to soil biota and the processes that result from their activity. Again this could be explained by a higher variation in biological soil parameters compared to abiotic parameters, and/or by soil processes being governed by different soil biota so that one-to-one relationships are less likely to become established. Hence, for soil quality assessment of grassland on sandy soils, the focus should be on abiotic parameters such as soil moisture content, SOM and total N. On other soil types these findings could be different. For the explanation of the N response to N fertilization, the mechanism behind the correlation between the number of enchytraeids and the N use efficiency has to be further investigated.

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Appendix. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.soilbio.2010.05.016.

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