Thesis dissertation:

Soil functioning underneath hedgerows in relation to adjacent land use, in a traditionally small-scale Dutch agricultural landscape.

Valerie L. Kalle
December 2019

WAGENINGEN UNIVERSITY & RESEARCH
NIOO
NEDERLANDS INSTITUUT VOOR ECOLOGIE (NIOO-KNAW)
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Page of content

Summary 1
1. Introduction 2
2. Methods 5
2.1. Study design 5
2.1.1. Study area description 5
2.1.2. Selection of fields and hedgerows 6
2.1.3. Soil sampling 7
2.2. Abiotic properties 7
2.3. Biotic properties 8
2.3.1. Microbial biomass 8
2.3.2. Mineralisation rates (in-situ experiment) 8
2.3. Hedgerow characteristics 9
2.4. Statistical analyses 10
3. Results 11
3.1. Abiotic properties 11
3.2. Biotic properties 11
3.3. Hedgerow characteristics 12
3.4. Interactions between abiotic and biotic soil properties, and hedgerow characteristics 12
4. Discussion 16
4.1. Comparison of soil properties among land use types 16
4.2. Influence of land use on soil properties underneath hedgerows 16
4.3. The hedgerow effect, and the influence of hedgerow characteristics on soil properties 17
5. Conclusion 18
Literature 19
I. Supplementary data: statistical results 22

Summary
Decennia of increasing agricultural efficiency has resulted in a global problem of soil degradation and impaired soil functioning. Hedgerow networks used to be inseparable of agricultural landscapes and contributed to their multi-functionality, but have disappeared with the movement of agricultural intensification. The main objective of this study was to determine the land use effect (conservation grassland, production grassland, and cropland), and hedgerow effect (hedgerow versus no hedgerow) on abiotic and biotic soil properties related to soil functioning. It was hypothesized that soil properties would be strongly affected by land use type, but that this land use effect would be less strong or even absent in hedgerows. The second objective was to identify differences in hedgerow characteristics (intactness, age, management type, etc.) among the three land use types. Lastly, the correlations between hedgerow characteristics, abiotic and biotic soil properties were studied. This study was conducted in the UNESCO Man- and Biosphere site called the Maasheggen, located in the Netherlands. Thus far, little is known about the soil properties of this Man- and Biosphere reserve. In total soil samples were collected from 18 fields and 35 hedgerows. These were analysed for abiotic properties (pH, soil organic matter (SOM), P-Olsen, nitrates, ammonium, soil organic carbon and nitrogen), and biotic properties (soil microbial biomass of bacteria, actinomycetes, fungi and arbuscular mycorrhizal fungi, and C and N mineralisation rates). Additionally, multiple characteristics of hedgerows were measured (age, species, intactness, height, width, understorey growth, buffer zone). The statistical analysis consisted of separate linear mixed models for all soil and hedgerow properties with the land use effect, hedgerow effect, and the land use · hedgerow effect as independent variables. Soil properties and hedgerow characteristics were tested for correlations, and multivariate analyses were performed to identify land use or hedgerow clusters. This study confirms the effect of land use on soil properties: i.e. lower SOM percentages and microbial biomass, and higher pH and P-Olsen values were found in croplands compared to conservation grasslands. Microbial biomass showed a positive correlation with SOM and a negative correlation with pH, and P-Olsen. The multivariate analysis showed a distinct cluster for hedgerows in soil properties compared to the other land use types. In hedgerows the land-use effect was significant for several soil properties: moisture content, pH, SOM, P-Olsen, ammonium, soil organic carbon, nitrogen, bacterial biomass, actinomycetes biomass, and lastly AMF biomass. However, the pattern observed for soil properties underneath hedgerows mostly did not correspond with the pattern observed among land use types. Instead, soil properties underneath hedgerows were found to correlate with the hedgerow characteristics age and intactness. Thus, I conclude that soil properties related to soil functioning underneath hedgerows were most similar to each other. The observed variability among hedgerows could partly be explained by the land use effect, but also by hedgerow characteristics related to the composition and state of the hedgerows.

1. Introduction

Over one-third of Earth's terrestrial land is designated as agricultural land, totalling ca. 45 million km^2 (Newcome et al., 2005; Ramankutty et al., 2008). Decennia of intensive farming has resulted in a global pressing issue of soil degradation involving both physical and chemical modifications of the soil (Govers et al., 2017). Nowadays, European soils are threatened by the loss of soil organic matter, soil biodiversity, erosion and landslides, soil compaction, soil sealing, contamination and salinization (EASAC, 2018). Many valuable services and goods to humans are provided by well-functioning soils, such as the provision of food, climate regulation and support of biodiversity (Dominati et al., 2010). Traditionally structural elements such as hedgerows were characteristic to agricultural landscapes, and contributed to the multi-functionality and heterogeneity of these man-made ecosystems (Forman & Baudry, 1984; Burel, 1996). From the 1950s onwards, many hedgerows disappeared during the process of rapid agricultural expansion and intensification (Kantelhardt et al., 2003). The question arises what the effect is of this loss of structural complexity on soil-functioning in agricultural landscapes.

Hedgerows together form a connected network in the agricultural landscape by separating or enclosing fields through rows of shrubs and/or trees (Pollard et al., 1974). Hedgerows are not fixed, but change over time depending on human activities, agricultural practices, history, ownership patterns, and geomorphology (Burel, 1996). Many hedgerows originate from the medieval period were they functioned as land boundary and as a fence for livestock. However, its importance vanished in the mid-nineties with the development of barbed-wire which replaced the natural fences, and mechanised agriculture which required larger parcels (Baltensperger, 1987; Baudry et al., 2000). Their importance have been described for climate regulation, erosion and water control, and to act as a corridor for plant and animal species (Burel, 1996; Baudry et al., 2000). Furthermore, hedgerows act as corridors and fulfil four main roles: habitat for species, acting as a barrier separating adjacent fields, source of abiotic and biotic influences on the adjacent fields, and lastly as a corridor for the movement of species (Forman & Baudry, 1984). The value of hedgerows to above-ground biodiversity within agricultural landscapes has been demonstrated in many studies performed on small mammals, birds, plants, and insects (Roy & de Blois, 2008; Silva & Prince, 2008; Wolton, 2015; Morandin et al., 2016; Heath et al., 2017). However, the belowground status of hedgerows in an agricultural landscape remains understudied.

A recent study performed in the United Kingdom by Holden et al. (2019) was the first to thoroughly examine soil functioning underneath hedgerows in an experimental agricultural landscape. These authors studied physical, chemical and a few biotic soil characteristics underneath hedgerows and field margins surrounding both arable and pasture land. The physical structure of hedgerow soils were found to be less compact, consisted of a higher fraction of micropores, and allowed for higher infiltration rates. Therefore, hedgerows were expected to have a positive effect on the water storage capacity within agricultural landscapes (Holden et al., 2019). Soil organic matter, nitrates- and phosphate concentrations were found to be greatest in hedgerow soils compared to pasture and cropland fields. However, their study on the soil microbiome was limited to total and arbuscular mycorrhizal fungal community in which they found a distinct cluster for hedgerow and arable soils.

Intensive high-input agriculture (e.g. conventional farming) generally achieves high yields through the use of fertilizers, herbicides and pesticides, high-yielding crop varieties, and mechanization (Knudsen et al., 2006; Hurni et al., 2013). It has been reported that plant species in hedgerows can be negatively affected by adjacent high-input agricultural fields, especially from fertilisation and pesticides drift (Tsiouris & Marshall, 1998; Deckers et al., 2004). Evidently, this has a great impact on soil functioning, which can generally be described by four main processes: transformation of carbon and formation of soil organic matter; nutrient cycling of main elements nitrogen, phosphor, and potassium; maintenance of soil structure by formation of biogenic structures; and lastly, safeguarding a stable soil community (Kibblewhite et al., 2007). These processes enable many of soils functions and services to humans such as the water quality regulation service, which is performed by soils as they filter and transform soil contaminants in soil water (EASAC, 2018).

Soil organic matter (SOM) is integral to understanding soil functioning as it links to soil's key functions (Milne et al., 2015). SOM is the combined input of organic compounds from plants, animals,

and microbes stabilized in clay minerals and soil aggregates (Lehmann and Kleber, 2015). A fraction of 2% SOM is considered the minimum threshold for maintaining soil's functions related to the physical structure, nutrient and pest regulation (Lal et al., 2016). On average, 50 to 58% of SOM is soil organic carbon (SOC) (Pribyl, 2010). SOC contains carbohydrates, simple sugars, complex organic compounds, some inert materials, and pyrogenic compounds (Lal et al., 2016). It holds unique properties, such as high surface area and charge density, which makes SOC a highly reactive compound to clay and other minerals. SOM pools shrink under conventional farming as a result of tillage, high mineral fertilizer input, residue removal, and by keeping soil bare for part of the year (Garratt et al., 2018).

The soil microbiome plays an important role in soil functioning (Bardgett & van der Putten, 2014), e.g. they are responsible for decomposition of dead organic matter and cycling of nutrients, which makes nutrients available to plants through mineralisation. A strong connection is found between the soil microbiome and SOM, as the stability of SOM strongly depends on the interactions of soil microbiota with the soil matrix (Cotrufo et al., 2013). This process is described in the ''*Microbial-Efficiency-Matrix-Stabilization''* (MEMS) framework developed by Cotrufo et al. (2013). It consists of two main processes: factors influencing the efficiency of the conversion of plant substrates into different by-products and microbial biomass, and interactions with the mineral soil matrix such as phyllosilicates, oxides, and calcium cations. Labile plant components from root exudates (i.e. fast-cycling SOM) play an important role in organic matter accumulation resulting from high microbial activity (Cotrufo et al., 2013). Thus, it is a reciprocal process as the soil microbiome stimulate SOM accumulation, and SOM also allows for a larger diversity of soil microbiota (Gardi et al., 2013; Garrat et al., 2018).

High-input, intensive agricultural practices with only low amounts of SOC are a major threat to the composition and biomass of the soil microbiome (Postma-Blaauw et al., 2010, Gardi et al., 2013). Frequent tillage is damaging to the soil microbiome as it disturbs the physical properties of soil and the microhabitats on which soil organisms depend (van Capelle et al., 2012). Land intensification has been shown to reduce diversity, abundance, and number of functional groups (Tsiafouli et al., 2015). This can be the result of high external inputs of nutrient, crop protection measures, soil tillage and the crop species used. A more sustainable agricultural production depends on less external inputs, chemical fertilizers and crop protection, and soil disturbance. Therefore, a more sustainable agriculture depends on a more substantial role of the soil microbiome for nutrient cycling, carbon cycling and storage, disease suppression, water infiltration and purification and lastly soil structure maintenance (Bardgett & van der Putten, 2014; Creamer et al, 2016).

The main objective of this study was to determine the land use effect (conservation grassland, production grassland, and cropland), and hedgerow effect (hedgerow versus no hedgerow) on abiotic and biotic soil properties related to soil functioning. The first objective was to identify differences among land use types, and how this translated to the soil properties underneath hedgerows. It was hypothesized that some negative effects of intensive agricultural practice may drift to the neighbouring hedgerows, thereby altering the soil functioning of these hedgerows. Intensive agriculture (i.e. croplands) were expected to have lower soil organic matter and carbon content, lower fungal and total biomass, and higher phosphate concentrations. Still, hedgerows were expected to cluster together than to its adjacent conservation grassland or production grassland or cropland. Following this, the second objective aims at identifying differences in soil functioning between hedgerows and the adjacent land use itself. Croplands were expected to be most different to hedgerows in soil's abiotic and biotic properties. Furthermore, general hedgerow characteristics were measured to determine its influence on soil properties. In croplands, hedgerows were managed as wild, proliferous hedges or as trimmed hedges, thus soil functioning of these cropland hedgerows were also tested for differences as a result of hedgerow management. Lastly, the relationship between abiotic and biotic properties was analysed, to identify the most important abiotic properties in explaining differences in the soil's microbiome.

The study area is called the 'Maasheggen' and is located in South-East Netherlands along the Meuse river. It is a traditionally small-scale hedgerow system that includes a variety of land uses in close proximity and is relatively well mixed. For this study soil sampling took place in the spring of 2019 in 18 fields and 36 hedgerows in the Maasheggen area, and the soil samples were then further

analysed in the laboratory. The following abiotic properties relating to soil functioning were measured: moisture content, pH, soil organic matter, plant-available phosphates, nitrate- and ammonium concentration, total nitrogen- and carbon content. For biotic properties, a fatty acid analysis was conducted to determine the total-, fungal-, AMF-, bacterial-, and actinomycetes biomass. Lastly, an in-situ experiment was performed to determine nitrogen and carbon mineralisation rates. These soil properties are also often measured in soil quality assessments (Schloter et al., 2003). This study was intended to contribute to understanding of soil functioning under hedgerows in order to provide knowledge on how to conserve hedgerows. We hope that the results may encourage follow-up studies on how to promote hedgerows in multi-functional agricultural landscapes.

2. Methods

2.1. Study design

2.1.1. Study area description

The Maasheggen is a unique study area because of its multi-functional cultural landscape, shaped by its hedgerows from historic small-scale agricultural practices, and nowadays includes a variety of land uses from conservation grasslands to high-input maize cropping in close proximity to each other (Benerink et al., 2011; Maasheggen, n.d.). It is located in the Netherlands along the Meuse stretching from Cuijk to Maashees. The total area encompasses 2000 ha, and involves a hedgerow network of at least 130 km (Maes et al., 2006; Maasheggen, n.d).

Nowadays, it is regarded as the oldest intact hedgerow system in the Netherlands (VNC, 2017). In the medieval period hedgerows functioned as land boundary and were used to fence-in cattle (Benerink et al., 2011; VNC, 2017). Hedgerows were maintained on a yearly basis through hedge-laying and trimming to prevent cattle from escaping. The small woody and shrubby species found in hedgerows are mostly thorny species such as blackthorn (*Prunus spinosa*), wild roses (*Rosa* spp.), hawthorn (*Crataegus mongyna*), and brambles (*Rubus* spp) or species such as ash (*Fraxinus excelsior*), elder (*Sambucus nigra*), and privet (*Ligustrum vulgare*) (Forman & Baudry, 1984). Three rare species are found commonly in the Maasheggen: buckthorn (*Rhamnus cathartica), Rosa balsamica,* and field maple (*Acer campestre*) (VNC, 2017). The soil is fertile because of riverine silt deposits that are also trapped within these hedgerow systems (Benerink et al., 2011). Three geomorphologically distinct soil types are present in the Maasheggen: the elevated levees with calcaric cambisol soils containing sandy loam, the lower river basins with gleyic fluvisol soils containing clay, and lastly the transition zone between these two soil types. The soils in the Maasheggen have been reported as calcium poor (Londo, 1968).

When traditional management practices were abandoned for agricultural intensification, also many hedgerows in this area disappeared (Londo, 1968; Benerink et al., 2011). Recently, in 2018 the Maasheggen has acquired an UNESCO Man- and Biosphere reserve status (Maasheggen, n.d.). The general aim of biosphere reserves is to achieve sustainable growth of both the natural environment and the local economies in that region (UNESCO, 1995). Partners of the Maasheggen (e.g. municipality of Boxmeer and Cuijk, Staatsbosbeheer, and Vereniging Nederlands Cultuurlandschap) have formed a collective ambition:*'' development of the Maasheggen as a connected nature reserve and UNESCO Man- and Biosphere reserve from a culture-historic, ecological, and recreation perspective, in which the development of green economies are stimulated''* (Uitvoeringsprogramma Noordelijke Maasvallei, 2016). This has led to enhanced efforts on conservation and restoration of hedgerows (Ministerie van Economische Zaken, 2015; Commissie Beheer Landbouwgronden, 1988).

The present study focuses on the region between Oeffelt and Beugen, called the 'Oeffeltse and Middelsteegse weiden'' (known as the ''Cultuurhistorisch monument'' in the region). This region still preserves many of the traditional, old, and intact hedgerow systems (Londo, 1968). Part of this area is designated as nature area and owned by the Dutch State Forestry Services (in Dutch: 'Staatsbosbeheer''). Many hedgerow systems next to this area are still in private property, and used for agricultural purposes (Peters et al., 2008). For example as production grasslands, maize croplands, beetroot croplands, or wheat croplands (Boer&Bunder, n.d.).

2.1.2. Selection of fields and hedgerows

Three different types of land use within the hedgerow systems were selected for this study: grasslands under nature conservation management (not fertilized, mown once or twice per year), intensively used production grasslands (high fertilizer input, frequent mowing), and croplands (Figure 2). These three types will be referred to as conservation grassland, agricultural grassland and cropland, respectively. The conservation grasslands have on average four species per m^2, and the main species found here are: red fescue (*Festuca rubra*), Yorkshire fog (*Holcus lanatus*), kinked foxtail (*Alopecurus geniculatus*), little clover (*Trifolium dubium*), sharp buttercup (*Ranunculus acris*), and lastly field sorrel (*Rumex acetosa*). The production grasslands typically consist of one grass species, i.e. perennial ryegrass (*Lolium perenne*). Maize is the main crop cultivated in croplands, except for one field where beetroot was cultivated. The plot size ranges from 0.35 to 2.32 ha, and the soil type varies from clayish (% lutite >25) to sandy clay (% lutite 17.5-25). For every land use category, six fields were selected that were comparable in terms of size, soil type, border and hedgerow type (except for cropland, see below). The most common shrub species found in the hedgerows were hawthorn (*Crataegus monogyna)*, blackthorn (*Prunus spinosa*), European spindle *Euonymus europaeus)*, dog rose (*Rosa canina)*, and common dogwood (*Cornus sanguinea)*. The hedgerows were all categorised as wild hedgerows, and can be seen as somewhat neglected. The description for these wild hedgerows is that they have not been trimmed for a long time, normally they are cut only every 20 to 25 years to one meter height (Maes et al., 2006). Most croplands have clipped hedgerows, meaning they are pruned to one meter height every year (Maes et al., 2006). For this study, three out of the six croplands had trimmed hedgerows.

Figure 1: Overview of the selected fields and hedgerows. Green circles: conservation grasslands. Yellow triangles: production grasslands. Light grey squares: croplands with trimmed hedgerows. Dark grey squares: croplands with untrimmed hedgerows.

2.1.3. Soil sampling

In total 18 fields with two adjacent hedgerows (6 fields per land use type), and 35 hedgerows (two hedgerows per field) were included in this study. Soil samples were collected at three locations: underneath two hedgerows and in the middle of the field (which is either conservation-, or production grassland, or cropland) (Figure 2). At every location, two soil cores up to 15 cm depth (two diameters: 1.5 and 3 cm) were collected at five sampling points. Those samples were pooled, thus forming a composite soil sample of ten soil cores for every location (Figure 2). Along the hedgerows a forty meter transect was laid out, with sampling points every ten meters. In the middle of the field, sampling points were set 10 meters apart and were connected through a W-structured transect. All soil sampling took place in 2019 on the 27th and 28th of May. Soil samples were kept in polyethylene bags in a cooling box, and after arrival at the institute they were stored at 4 °C (Gregorich & Carter, 2006). The preparations for long-term storage of soil for all chemical analyses and fatty acid analysis for microbial biomass took place within a week after soil collection in field.

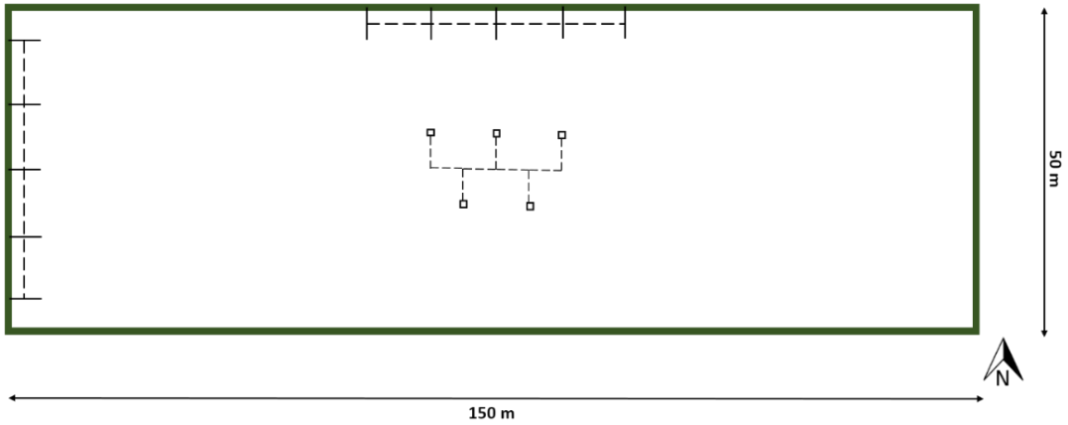

Figure 2: Sampling design used for collecting soil samples. Underneath two hedgerows (green) five sampling points were set in a forty meter transect, and in the middle of the field five sampling points were set out in W-shaped transect.

2.2. Abiotic properties

SOM was determined by the loss-on-ignition method, and SOC with nitrogen fraction was determined by the micro-Dumas method. (Storer, 1984; Stewart, 1964; Suehara et al., 2001). For the LOI-method 5 to 10 g of soil was first dried at 105 °C for 24 hours, and subsequently placed in a muffle furnace at 430 °C for 24h. The difference in weight is then expressed as the percentage SOM. For the micro-Dumas method between 3000 to 5000 µg 40°C oven-dried, and grinded soil was weighed and wrapped into small tin-foil capsules. The Flash EA 112 Elemental Analyser was used to measure total organic carbon by which oxidation occurs in the combustion reactor at 1800 °C, after which the gases were quantified as percentage C in the thermal conductivity detector. All soil samples had a pH below 7.8, therefore it can be assumed that little inorganic carbon is present (Dhillon et al., 2015). Nitrates (NO_3+NO_2) and ammonium (NH_4) in the soil were extracted from 10 g using 1 M KCl, and the amounts were measured at certain wavelengths (520 nm, and 660 nm) using an Auto Analyser (*SEAL QuAAtro Segmented Flow Analysis (SFA) system)* (Keeney & Nelson, 1982). The sum of NH_4+NO_x was indicated as total N. The sodium bicarbonate extraction method was used to determine the plant-available phosphorus (P-Olsen) content using 2.50 g of dry soil (Olsen, 1954), and was measured using the Auto Analyser. Lastly, standard measurements such as moisture content (%) and pH-H_2O were performed.

2.3. Biotic properties

2.3.1. Microbial biomass

A phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) analysis was performed to determine the soil microbial biomass. This procedure consists of three steps using freeze-dried soil: first the extraction of total lipids, second the division into fractions of different polarity, and last the formation of fatty acid methyl esthers. The PLFA and NLFA concentration were measured by gas chromatography using the FAME and BAME as reference standard mixes (Frostegård et al., 2011).

For bacterial biomass the following PLFA markers were used: iC15:0; aiC15:0; C15:0; iC16:0/#C16:4w3; #C16:3w3/#C16:1w9c; C16:1w7c/#C16:1w9c; C16:1w6c/#C16:1w7t/#C16:2w4 pufa 3; iC17:0; #aiC17:0; cy-C17:0; C17:0; C18:1w9t/C18:1w7c; cy-C19:0 (Frostegård et al., 1993; Frostegård & Bååth, 1996). The PLFA marker C18:2w6c was used for the determination of fungal biomass (Federle, 1986). To determine arbuscular mycorrhizal fungal biomass the NLFA marker C16:1w5c+t was used (Hedlund, 2002). Lastly, the PLFA markers #10MeC16:0; #10MeC17:0; #10MeC18:0 were used as a proxy for the biomass of actinomycetes (Frostegård et al., 1993). The sum of all PLFA's was used as a proxy for total microbial biomass and biomass is expressed in µg C ·g dry $soil^{-1}$.

2.3.2. Mineralisation rates (in-situ experiment)

The nitrogen and carbon mineralisation assay was set-up approximately three months after sampling in the field (September to November 2019). For both assays the samples were kept at 65% water-holding capacity (relates to an average moisture content of 26%), and incubated in the dark at 18 °C and 100% humidity. The water-holding capacity was measured by placing 1 to 5 g soil on a filter paper and adding water up to the point where all soil was submerged (Bradford Lab Protocol, 2010a). Then the samples were drained for two hours and the moisture content was determined. Prior to the start of trial, the moisture content of all samples was again measured, followed by bringing the moisture content to 26%. Approximately 25 g of soil was incubated for 23 days for the N mineralisation assay. The moisture content was checked three times during the incubation period. The 1M KCl extraction procedure was performed in order to determine the nitrate and ammonium contents before- and after incubation (Keeny & Nelson, 1982). Thus, the potential N mineralisation rate was expressed as the weekly increase in mineral N (nitrates+ammonium) (van Eekeren et al., 2010).

The C mineralisation rate was averaged from two measurements using the gas chromatograph and is expressed in C mg· kg dry $soil^{-1}$· day^{-1} (Bradford Lab Protocol, 2010b). In a 50 mL tube around 4 to 5 g soil was weighed and put at a 26% moisture content. Then the soil was adjusted to the incubator environment for at least one day before actual measurements started. The tubes were flushed with nitrogen for three minutes at 2 bar, and then incubated for 24 hours. After 24 hours a mixed sample of 12 mL was taken under positive atmospheric pressure and injected into a flushed and evacuated exetainer for automated measurements in the gas chromatograph (CO_2-IR, Autosampler). The injection volume was 250 µL, the column temperature was 50 °C, and the process took 90 seconds per sample. The ideal gas law was used to calculate the carbon mineralisation rate (see calculation below). The total atmospheric pressure was set at 101325 Pa, the universal gas constant at 8.31 $m^3 \cdot Pa \cdot K^{-1} \cdot mol^{-1}$, and temperature at 298 K.

(1) Conversion of CO_2 from ppm to mg/mL:

Total atmospheric pressure · (CO_2 concentration/10^6) *divided by*

(Universal gas constant * Temperature · Molar mass of CO_2)/ 1000

(2) Calculation to carbon mineralisation in C mg· kg dry $soil^{-1}$· day^{-1}

CO_2 (mg/mL) · headspace volume (mL) / soil dry weight (g) ·1000 / time (h)

Multiplied by fraction of C

2.3. Hedgerow characteristics

All hedgerows in this study belonged to the category proliferous, untrimmed hedgerows, except for five trimmed hedgerows surrounding three croplands. In October 2019 all hedgerows in this study were inventoried to determine the status of these hedgerows based on a few characteristics described in the study by Maes et al. (2006). Hedgerows were assessed for their age, intactness, shrub species richness and lastly their height and width. Furthermore, information was collected on the length of the buffer zone, and the understorey vegetation growth was visually assessed. A forty meter transect parallel to the hedgerow was set up to collect all information, starting from the middle of the hedgerow and then twenty meters to each side. Intactness was measured as the fraction of present hedges in a forty meter transect. Both age and understorey growth were visually assessed. Age consisted of three age classes: class 1: young; class 2: middle-aged; and class 3: old, Figure 3 shows the hedgerows that were used as reference. Understorey vegetation growth was also grouped into three classes: class 1: bare soil to low vegetation growth; class 2: medium vegetation growth of species such as stinging nettle (*Urtica spp.*); and class 3: tall vegetation of species such as blackberry bushes (*Rubus spp.*) (Figure 4). For every class the percentage in the forty meter transect was scored, from this the weighed mean was calculated for both hedgerow properties.

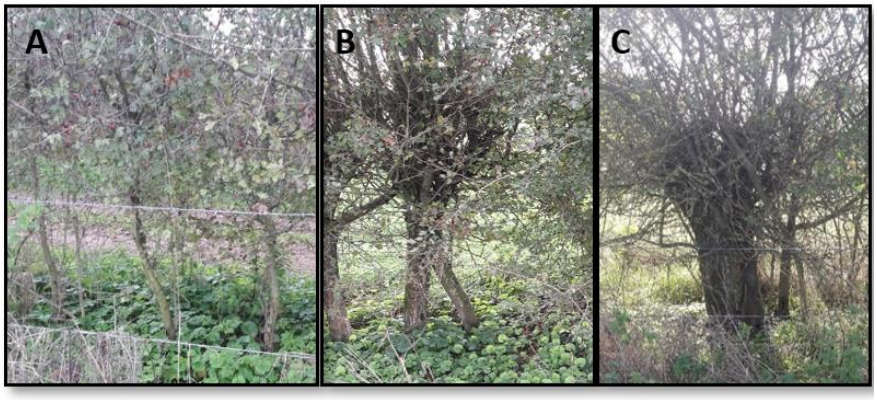

Figure 3: The visual classification of trees into three age-classes (A) young, (B) middle-aged, (C) old trees.

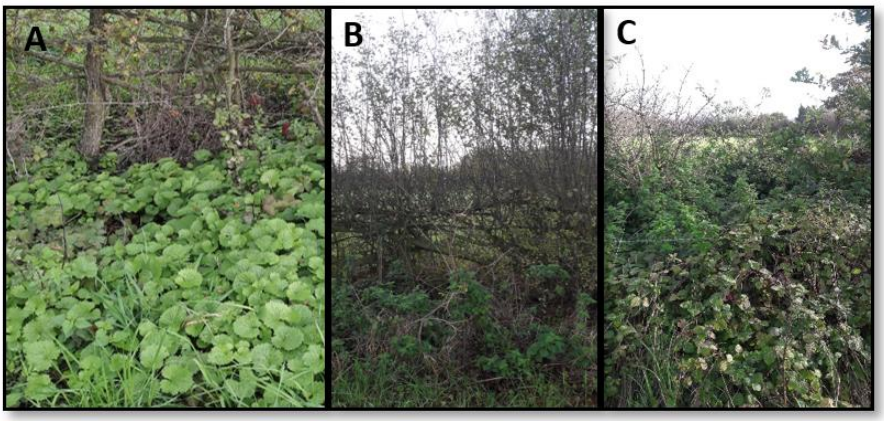

Figure 4: The visual classification of understorey vegetation growth into three categories (A) bare to low vegetation growth, (B) medium vegetation growth, (C) high vegetation growth.

2.4. Statistical analyses

Linear mixed models (LMM) were used to test for differences in hedgerow characteristics, abiotic and biotic properties between land use types (the land use effect) and hedgerows versus no hedgerows, i.e. fields (the hedgerow effect). This was performed for each soil property separately with the land use effect, hedgerow effect and, the land use · hedgerow effect as fixed factors, and field code as random factor. Furthermore, significant differences within and between fixed factors groups were tested with a LSD post-hoc analysis. Different analyses were conducted for a few properties: moisture content, C mineralisation, log(fungi), height, width, and understorey vegetation growth. For moisture content a LMM was performed with both field code and sampling day as a random factor. For C mineralisation, height, width and understorey vegetation growth, non-parametric tests were used, thus separately testing the land use effect (Kruskal-Wallis) and hedgerow effect (Mann-Whitney). To find differences between land use types, the combinations were tested separately with a Mann-Whitney non-parametric analysis. The log(fungi) did not show a positive final hessian matrix in the LMM, thus a separate ANOVA univariate analysis was performed with a LSD post-hoc analysis. In croplands management types (trimmed versus untrimmed hedgerows) were compared in LMM test to adjust for field code. Moisture content, log-pH, N mineralisation rate, shrub species were tested for differences between management types with an Independent T-Test as no positive final hessian matrix was found in the LMM.

Correlations between soil properties were tested for all soils and soils from hedgerows only, here the Pearson correlation coefficient was used or the Spearman rank test for the non-normal distributed properties. The separate analysis performed on hedgerows also included hedgerow characteristics. A principal component analysis (PCA) and corresponding redundancy analysis (RDA) with 499 permutations were performed to get more insight into the relationship between variables and to identify variables that were responsible for most variation observed in the dataset. For this analyses a combined variable of land use and hedgerows was constructed, thereby creating 7 categories: conservation grasslands, conservation grasslands · hedgerows, production grassland, production grasslands · hedgerows, cropland · trimmed hedgerows, cropland · wild hedgerows. Four different models were set-up (Table S7): (1) soil properties explained by land use, (2) soil properties underneath hedgerows explained by land use and hedgerow characteristics, (3A) microbial biomass explained by abiotic properties, (3B) microbial biomass explained by both abiotic properties and land use, (4) microbial biomass underneath hedgerows explained by abiotic properties, land use, and hedgerow characteristics.

Prior to analyses all continuous properties were tested for normality per land use type. This was not found for several properties. Multiple outliers were identified for several soil, and hedgerow properties, and are shown in Table 1 (defined by SPSS as 1.5×Interquartile range). Some properties were log-transformed to fit model's assumptions: pH, P-Olsen, fungi, AMF, and age. This was not possible for C mineralisation rate, height and width and understorey growth, and these were tested with non-parametric tests. All statistical analyses were performed in SPSS 25 (IBM Corp., 2017), and CANOCO 5 (Šmilauer & Lepš, 2014).

Table 1: Overview of all soil properties with outliers split by land use type and hedgerow versus field. It shows the number of outliers and whether it was positive or negative to the mean of that group (defined by SPSS as 1.5×Interquartile range). The following abbreviations were used: NMIN.: N mineralisation, CMIN.: C mineralisation, BAC.: bacterial biomass, ACT.: actinomycetes biomass, FUN.: fungal biomass; TMB: total microbial biomass, SP: shrub species.

	pH	SOM	P	NO_X	NH_4	Total N	NMIN.	CMIN.	BAC.	ACT.	FUN.	AMF	TMB	HEIGHT	WIDTH	SP
Outliers (nr)	5	3	2	4	1	1	2	1	2	1	4	2	2	2	1	1
Positive	2			1		1	1	1	1	1	4	2	2			
Negative	3	3	2	3	1		1		1					2	1	1

3. Results

3.1. Abiotic properties

The land use effect (conservation-, and production grassland, and cropland) was significant for all abiotic properties apart from moisture content (Table S1). The hedgerow effect (hedgerow versus no hedgerow) was not significant for the abiotic properties moisture content, pH, and total NH_4+NO_x. The interaction between the land use · hedgerow effect was only not significant for the abiotic properties pH and NH_4. Croplands differed from both production- and conservation grasslands in pH, SOM, C, and N (Figure 5, Table S3). The pH was highest in croplands, but SOM, C, and N were lowest. The P-Olsen concentration was significantly highest in croplands, followed by production grasslands, and lowest in conservation grasslands. The NO_x concentration was significantly lower in conservation grasslands than in both cropland and production grasslands. The NH_4 concentration was lower in croplands than in production grasslands. Hedgerow soils near conservation grasslands had a higher moisture content than those near croplands (Table S3). Cropland hedgerow soils were significantly different to both production- and conservation grasslands for pH and only to conservation grasslands for P-Olsen concentrations (Figure 5). Production grassland hedgerows had significantly higher percentages of SOM, C, and N, and higher concentrations of NH_4 than hedgerows near both croplands and conservation grasslands. The hedgerow effect was present in croplands, with higher percentages SOM, C and N, and NH_4 concentrations in the adjacent hedgerows than in croplands, whereas the opposite was found for moisture content and NO_x concentrations (Table S3). In production grasslands, there was a significant hedgerow effect for NO_x and NH_4 concentrations, showing similar patterns as in croplands. In conservation grasslands, the hedgerow effect was found for SOM, P-Olsen and NH_4. The adjacent hedgerows had higher P-Olsen and NH_4 concentrations, but lower SOM percentages. There were several significant correlations between abiotic properties (Table S5). SOM was negatively correlated with pH and P-Olsen, and positively correlated with % C, % N and NH4. Apart from SOM, pH also showed a positive correlation with P-Olsen and a negative correlation with NH4, % C, and % N.

3.2. Biotic properties

There was a significant interaction of the land use · hedgerow effect for all soil microbial taxonomic groups (bacteria, actinomycetes, fungi, AMF, and total) (Table S1). Bacterial-, and actinomycetes, and total microbial biomass were lowest in croplands, than higher in production grasslands, and highest in conservation grasslands (Figure 6A, Table S3). Fungal and AMF biomass were highest in conservation grasslands than in both croplands and production grasslands. In hedgerows, bacterial and actinomycetes biomass were significantly higher next to conservation grasslands than croplands (Figure 6B, Table S3). The AMF biomass was higher in hedgerows near croplands than production grasslands. In croplands the biomass of all microbial groups were significantly higher in the adjacent hedgerows (Table S3). In production grasslands only actinomycetes-, fungal- and AMF- biomasses were greater in hedgerows. The opposite was found for conservation grasslands were the biomass of all microbial groups was greater than in the adjacent hedgerows. All microbial biomass groups were significantly correlated ($P<0.01$) (Table S5). N mineralisation rate showed a significant interaction with the land use · hedgerow effect (Table S1). In production- and conservation grasslands, N mineralisation rate was greater in than in croplands (Table S3). Only lower N mineralisation rates were found in croplands compared to the adjacent hedgerows. There was no influence from the land use, hedgerow, and land use · hedgerow effect on C mineralisation rate. Only total microbial biomass showed a positive correlation with N mineralisation rate (Table S5).

3.3. Hedgerow characteristics

Apart from hedgerow width, there was no overall significant effect of land use on hedgerow properties (Table S2). Hedgerows near croplands were smaller than hedgerows near conservation-, and production grasslands (Table S4). Significant differences were found for intactness and age, however only in a two-way comparison between hedgerows near production- and conservation grasslands. The older and less intact hedgerows were found near production grasslands. In croplands two types of hedgerow management were compared (clipped versus wild hedgerows). These two types of hedgerows differed only in moisture content and height ($P<0.01$). As expected, clipped hedgerows were less tall than wild hedgerows, and had a higher moisture content (Table S4). However, soils under both hedgerow types were collected at two different field sampling days. Understorey vegetation growth showed a positive correlation with age and species (Table S6). A negative correlation was found between intactness and buffer zone, and between intactness and species.

3.4. Interactions between abiotic and biotic soil properties, and hedgerow characteristics

Land use explained 48.8% of total variation in soil properties in a model containing data from both land use itself and adjacent hedgerows. Most of the observed variation was explained by cropland and conservation grassland, respectively 22.9% and 14.6% (Figure 7, Table S7). When focussing on the soil microbial taxonomic biomass groups (bacteria, actinomycetes, fungi, AMF, and total), soil abiotic properties alone explained 54.1% of total variation. The most important soil properties that were found were: SOM (31%), P-Olsen (12%), log(pH) (7%), moisture (5%), and lastly NO_x (3%). After addition of land use to this model, SOM (31.1%) remained the most important explanatory variable, followed by conservation grassland (18%), cropland (8%), P-Olsen (4%), pH (4%), and production grassland hedgerows (2%). Accordingly, a significantly positive correlation was found between SOM and biomass for all microbial groups except AMF (Table S5), and a negative correlation was found between SOM and log-pH and P-Olsen. In a model containing data from hedgerows only, the combination of land use and hedgerow properties explained 28.7% of the observed variation in soil properties. Wild hedgerows near croplands, followed by intactness explained most of the variation (25%). In hedgerows, most variation in soil microbial biomass was explained by moisture content (17%), P-Olsen (8%), log(pH) (8%), and log(age) (6%). Accordingly in hedgerows, there was a significantly positive correlation between total microbial biomass and moisture content, and there was a negative correlation with P-Olsen (Table S6). Similar patterns were found for bacterial-, and actinomycetes biomass. AMF showed a positive correlation with pH and a negative correlation with SOM. Hedgerow age showed a positive relationship with SOM, C, N and fungal biomass. Furthermore, intactness showed a negative relationship with SOM, NO_x, NH_4, C, and N. In contrast, hedgerow age showed a positive relationship with SOM, C, N and fungal biomass (Table S6).

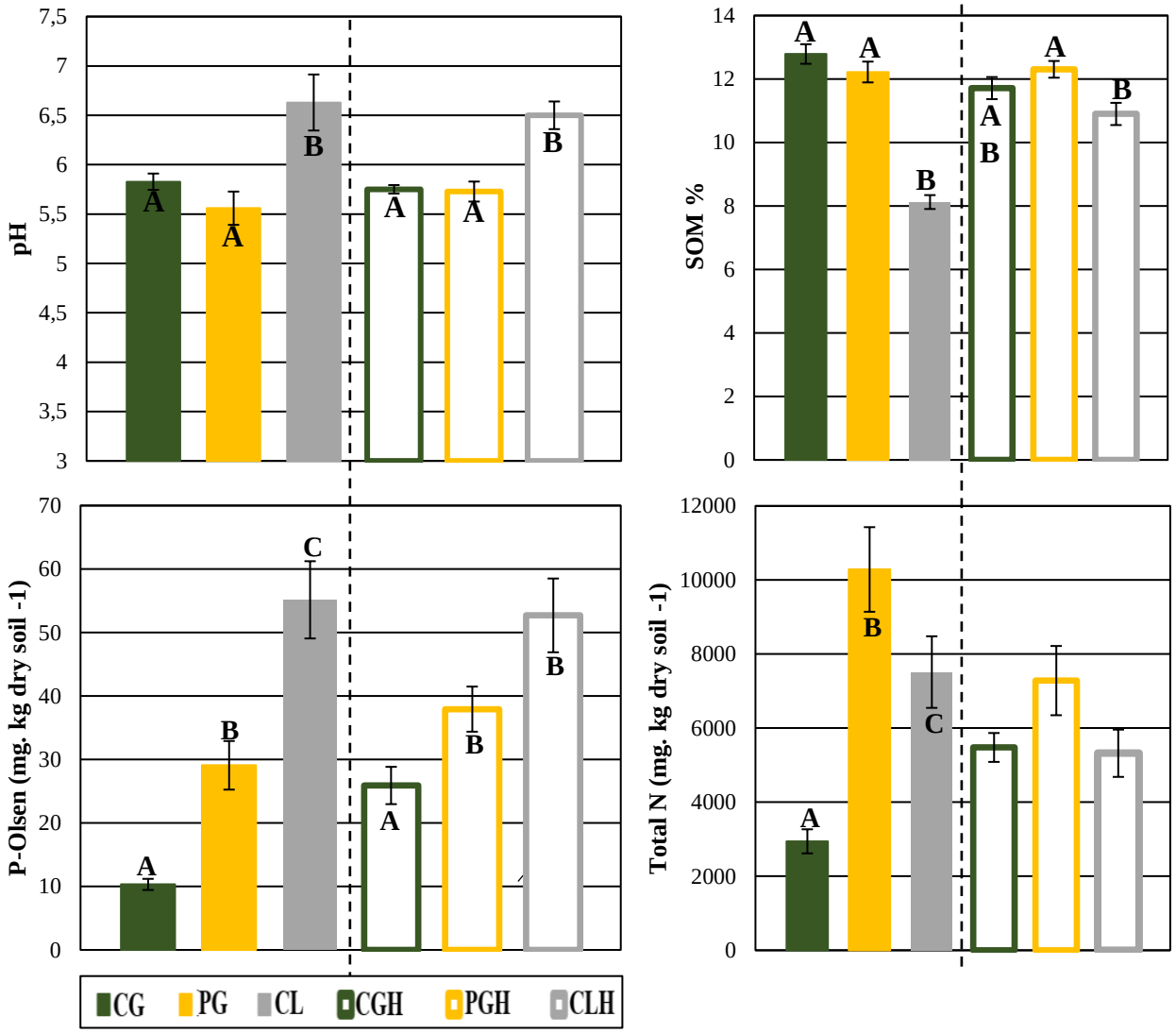

Figure 5: Abiotic properties for the three land use types (conservation-, and production grasslands, and croplands) from no hedgerow and hedgerow soils. CG: conservation grasslands, PG: production grasslands, CL: croplands, CGH: hedgerows near conservation grasslands, PGH: hedgerows near production grasslands, CLH: hedgerows near croplands. Different letters indicate significant differences ($P<0.05$) between land use types for no hedgerow and hedgerow soils.

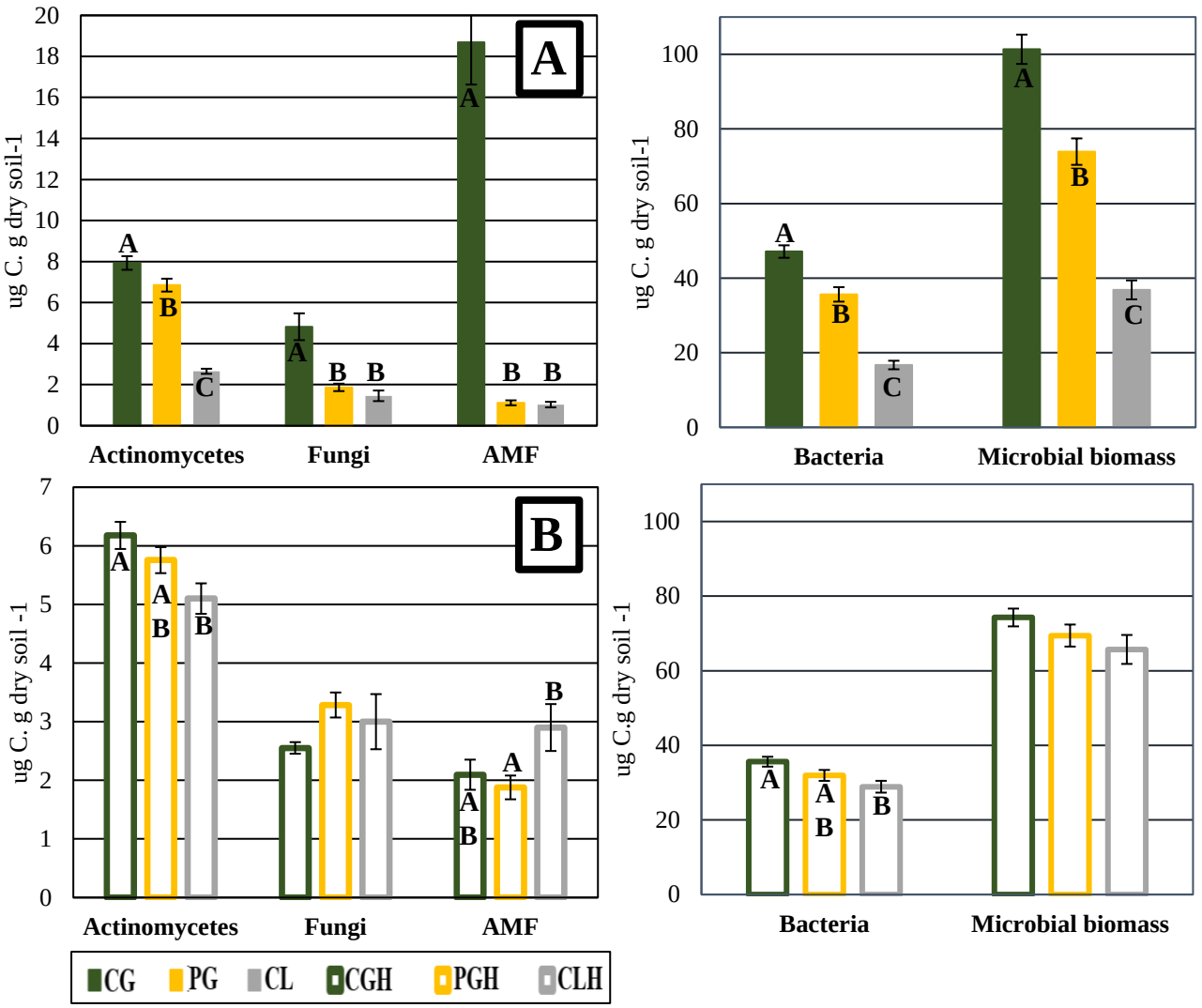

Figure 6: Taxonomic microbial biomass groups for the three land use types (conservation-, and production grasslands, and croplands), separately for no hedgerow (A) and hedgerow (B) soils. CG: conservation grasslands, PG: production grasslands, CL: croplands, CGH: hedgerows near conservation grasslands, PGH: hedgerows near production grasslands, CLH: hedgerows near croplands. Different letters indicate significant differences ($P<0.05$) between land use types for no hedgerow and hedgerow soils.

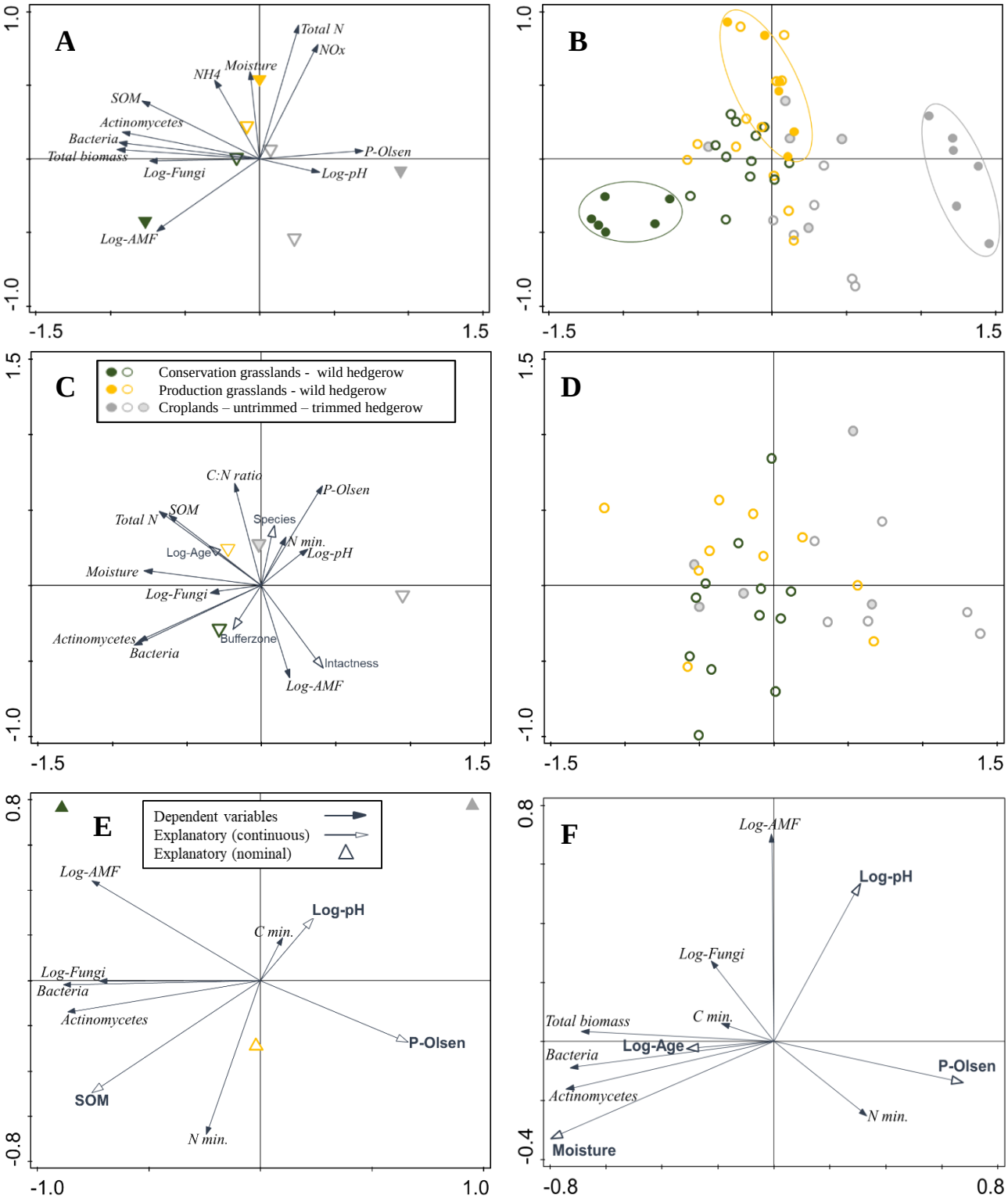

Figure 7: Graphs from PCA (A-D) and RDA (E-F) results. Model 1 (A-B): effect of land use on soil properties. Model 2 (C-D): effect of land use and hedgerow properties on soil properties, only in hedgerows. Model 3 (E): soil microbial biomass explained by land use and abiotic properties. Model 4 (F): soil microbial biomass explained by land use, abiotic and hedgerow properties, only in hedgerows. Additional results from analyses can be found in Table S7.

4. Discussion

4.1. Comparison of soil properties among land use types

Croplands had lower percentages SOM, C and N, thereby confirming earlier work showing that intensive agricultural practices deplete the soil's organic matter reservoir (Garratt et al., 2018). In the present study croplands were also characterised by a higher pH, P-Olsen, and a lower NH_4 concentration compared to production- and conservation grasslands. Only NO_x values did not differ between croplands and production grasslands, whereas these values were lowest in conservation grasslands. The explanation for the observed higher NH_4 concentration in conservation grasslands may be a better adsorption capacity of the soil. NO_x is more sensitive to leaching, and fertilisation can increase NO_x concentrations in croplands and production grasslands, where this fertilizer is applied most excessively (Rao & Puttana, 2000). Phosphorous has been accumulating in Dutch agricultural soils with surpluses of 25-30 kg· ha^{-1} (Smil, 2000; Tunney et al., 2003), as a result of excessive fertilisation schemes that aim at high crop yields (Tóth et al., 2014). This agrees with the findings in the present study: i.e. more than five times the P-Olsen concentrations were found in croplands compared to nearby conservation grasslands. Croplands are often limed to increase phosphor availability to plants which increases when pH is above 5.5 (Mahler & McDole, 1987), this was also observed in the present study.

Intensive agricultural practices interferes with soil's abiotic properties, especially with pH, SOM and total phosphor (Delgado-Baquerizo et al., 2017; Plassart et al., 2019). This in turn may alter a soil's microbiome, which has already been observed for bacterial diversity and community composition in a study by Delgado-Baquerizo et al. (2017). In the present study, total microbial biomass was almost three times higher in conservation grasslands than in croplands. Similar patterns were found for bacteria, actinomycetes, fungi and AMF. Most prominent was the relatively high biomass of AMF in conservation grasslands. Microbial biomass has been linked to land use, where changes from unmanaged to agricultural systems showed a decrease in microbial biomass as a result of decreased plant C inputs (Franzluebbers et al., 2000). This agrees with our multivariate analyses, namely the variance in soil properties were mostly explained by the two contrasting land uses: croplands and conservation grasslands. Furthermore, most variation in biomass among the different soil taxonomic microbial groups were explained by the same abiotic properties: SOM > P-Olsen > pH. Delgado-Baquerizo et al. (2017) reported found a negative standardized path coefficient between microbial biomass and total P (-0.70) and a positive path with SOM (0.28). Analogous, the present study found correlations between biomass and SOM, and P-Olsen for all taxonomic microbial groups except for AMF.

Microbial activity in terms of carbon and nitrogen mineralisation rate, is determined by microbial diversity or soil organic carbon (Tardy et al., 2015). In the present study no effect of land use was found on carbon mineralisation rate. This may be a shortcoming of the low level of repetition used in this experiment (only repeated twice). However, the lowest N mineralisation rate was found in croplands that also had the lowest soil organic carbon fraction, which determines microbial activity through enhancing soil microbial biomass (Tardy et al., 2015). Faster N mineralization rates have been linked to lower fungal to bacterial ratio, or lower fungal biomass (Högberg et al., 2007; de Vries et al., 2006). In the present study, croplands were characterized by both lower fungal- and bacterial biomasses, however, not by lower fungal to bacterial ratios. Bacterial dominance in the soil microbiome has been found to increase N mineralization rates (Orwin et al., 2019).

4.2. Influence of land use on soil properties underneath hedgerows

The strong impact observed of land use on soil properties, was only small in hedgerows. Soil properties of the hedgerows were significantly influenced by adjacent land use with respect to moisture content, pH, % SOM, P-Olsen, NH4, % C, % N, bacterial biomass, actinomycetes biomass, and AMF biomass. This may suggest a wider effect of land use on soil properties in hedgerows. However, no corresponding pattern among land use types and among hedgerows were found for several of these soil properties. The moisture content was less underneath hedgerows near croplands than conservation grasslands, an explanation for this is that many hedgerows near croplands were elevated and thus collecting less water

after a rainfall. SOM was lower underneath hedgerows near croplands than in production grasslands. However, this does not correspond completely with the pattern found among land use types as SOM was significantly higher in conservation grasslands, than in production grasslands, and lowest in croplands. The AMF biomass was higher in hedgerows near croplands than near production grasslands. In contrast, conservation grasslands were found to have substantially higher AMF biomass than in both production grasslands and croplands. Thus, variability in soil properties among hedgerows may not be explained by adjacent land use alone, but may possibly be affected by other variables that can be linked to land use, such as history of the hedgerow, a soil's physical properties, and herbaceous plant species.

In the present study significant differences in P-Olsen concentrations were found among all land use types, whereas in hedgerows only a trend was observed among all land use types. Only, hedgerow soils near conservation grasslands showed significantly lower P-Olsen concentrations than near both production grasslands and croplands. It has already been suggested that fertiliser application in the field can enter hedgerows (Pollard et al., 1974; Forman & Baudry, 1984). Similar to the study by Holden et al. (2019), higher pH levels were found in both croplands and the adjacent hedgerow soils compared to the other land use types. Higher bacterial- and actinomycetes biomass were found underneath hedgerows near conservation grasslands than in croplands, this correlated with hedgerow soils with a higher moisture content, higher SOM, higher total NH_4+NO_x, and lower P-Olsen concentration. In hedgerows variations in soil microbial biomasses were mostly explained by moisture content followed by P-Olsen, pH and age of the hedgerow. A study by Delgado-Baquerizo et al. (2017) found that bacterial diversity peaked under environmental conditions linked to intensive agricultural practices (high P availability, low SOM, low C:N, N:P, and C:P ratios). Croplands were able to support a-Proteobacteria and Firmicutes in soil microbial communities (Delgado-Baquerizo et al., 2017). Interestingly, no relationship has been identified between bacterial diversity and microbial biomass (Delgado-Baquerizo et al., 2017). Although microbial biomass proved to be a good indicator for land use-driven changes in soil properties, it does not serve as a proxy for a soil's microbial diversity and community. Therefore, it is recommended to study this in hedgerows, as it has also been linked to microbial activity patterns that are responsible for many soil processes (Tardy et al., 2015). It may be that the effects of adjacent land use on hedgerow soil microbial communities are negligible because soil microbes limited dispersal capacity, this was shown in bacteria in a study by Chemidlin Prévost-Bouré et al. (2014)

4.3. The hedgerow effect, and the influence of hedgerow characteristics on soil properties

Hedgerows, regardless of land use type, clustered together in the PCA performed on all soil properties in the present study. Soils underneath hedgerows have been recognised to be distinct of both arable land and pastures; this applies to a number of soil properties related to either soil quality or soil functioning (Monokrousos et al., 2006; Holden et al., 2019). Holden et al. (2019) found highest nitrate and phosphate concentrations in hedgerows regardless of adjacent land use. In the present study we have also found higher concentrations of P-Olsen in hedgerow soils than in conservation grasslands. Regardless of adjacent land use, NH_4 concentrations were higher in hedgerow soils. This was not the case for NO_x concentrations, which was higher in both croplands and production grasslands than in hedgerows. However, no differences in nitrate concentrations between hedgerows and croplands were found in a study by Monokrousos et al. (2006). Holden et al. (2019) explained this observed trend of higher pollutant concentrations in hedgerows compared to adjacent land use as following: a lower moisture content in soils underneath hedgerows may result in a more concentrated soil solution, and dry deposition through canopy leaching may be enhanced in hedgerow soils (Gallagher et al., 2002). The lower NOx concentrations in hedgerows compared to production grassland and croplands found in the present study, could be explained by faster nitrate uptake by trees compared to bare soil in cropland and grasses in production grasslands, as was reported by Grimaldi et al. (2012) who studied hedgerows that were classified as a row of oak trees. These differences in soil properties found in hedgerows among studies may be the result of site-specific characteristics such as: landscape topography, soil type, river flow synchronisation effects, and amount of applied fertilisation (Rogger et al., 2017).

The least significant differences between hedgerows and adjacent land use were found for production grasslands, which only differed for NOx and NH4 concentrations for the abiotic properties. Also, no differences between hedgerows and production grasslands were found for bacterial and total

microbial biomass. The similarities shown between pastures and forests have already been reported in a study by Franzluebbers et al. (2000), they showed that pasture and forest land were similar in their carbon storage capacity. However, soil microbiomes of forests have been identified as a distinct cluster compared to other land use types in a study on bacterial communities (Plassart et al., 2019), and a study on the total soil microbial communities (Mendes et al., 2015). According to Mendes et al. (2015), forest soil ecosystems are maintained by high microbial abundances, because these systems are more stable they have lower microbial diversity, and functional redundancy than in disturbed, agricultural systems. The present study found correspondingly higher biomass of all soil microbial taxonomic groups in adjacent hedgerows than in croplands, whereas the opposite was found in conservation grasslands. This also supports the findings by Constancias et al. (2015) that forest soils support higher soil microbial biomasses than soils of croplands.

Overall, no significant effect of adjacent land use was found on hedgerow characteristics apart from height. When comparing only hedgerows near production- and conservation grasslands, a few hedgerow characteristics were found to be significantly different: intactness and age. Hedgerows near production grasslands were older, but less intact than hedgerows adjacent to conservation grasslands. Possibly, more gaps are present between older trees than the younger, more recently planted trees. Soils of hedgerows near production grasslands were associated with higher SOM and fungal biomass compared to the other land use types. Interestingly, the age of hedgerows showed a positive correlation with SOM and fungal biomass, whereas intactness was negatively correlated with % SOM, total NH_4+ NO_x, % C and % N. In croplands the two hedgerow types were pooled together, because no differences in soil properties were found between clipped versus unclipped hedgerows. Multiple studies have focussed on the herbaceous understorey layer in hedgerows, but the present study limited species richness to shrubs (Forman & Baudry, 1984; Kantelhardt et al., 2003; Deckers et al., 2004; Roy & de Blois, 2008; Sitzia et al., 2014). Land use has been found as the most important regulator of species richness in hedgerows, after hedgerow management, soil conditions, hedgerow type and dimensions (Deckers et al., 2004). Sitzia et al. (2014) found that distinct vegetation clusters were also different in topsoil organic matter properties. Thus, the observed variability in soil properties among hedgerows adjacent to the three land use types may be affected, at least in partially, by differences in understorey vegetation composition and richness.

5. Conclusion

To return to the research questions of the present study, land use showed a strong effect on soil properties, soil properties differed between hedgerows and adjacent land use, and the effect of nearby land use on soil properties was less strong and different underneath hedgerows. The abiotic properties of cropland soils differed from both grasslands, and the biotic properties of conservation grassland soils differed from both production grassland and cropland. Variability in soil microbial biomass was mostly explained by the abiotic properties SOM, P-Olsen, and pH in all soils, and by moisture content, P-Olsen, and pH in hedgerow soils. Production grasslands showed least significant differences in abiotic and biotic properties with their adjacent hedgerows. Croplands had consistent lower soil microbial biomass than in hedgerows, whereas the opposite was found in conservation grasslands. Overall, no effect was found of land use on hedgerow characteristics, but specific significant interactions were found between production grasslands and conservation grasslands for intactness and age. Hedgerow characteristics did show some interesting correlations with soil properties: age showed a positive correlation with SOM and fungal biomass, intactness a negative correlation with SOM, and total NH_4+ NO_x.

The present study showed that soil properties relate to soil functioning underneath hedgerows may be localized and can be maintained regardless of adjacent land use type. However, more research is needed into understanding variability in soil properties underneath a single hedgerows, and how hedgerows contribute to ecosystem services delivered by agricultural landscapes. Thus, it is essential to further study the importance of hedgerows in achieving a multi-functional agricultural landscape.

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I. Supplementary data: statistical results

Table S1: Output of statistical analyses with soil properties and hedgerow characteristics as dependent variables. The land use effect (conservation-, production grassland or croplands), the hedgerow effect (no hedgerow versus hedgerow) and the land use · hedgerow effect as independent variables. Linear mixed models (LMM) were performed for most soil properties with field code as a random factor, showed by the degrees of freedom numerator (dfn) and denumerator (dfd), F-values and P-values. Some properties were tested with non-parametric tests (Kruskal-Wallis for land use, and Mann-Whitney for H vs. F effect), this is indicated by NP in brackets. For the Kruskal-Wallis test degrees of freedom (df) with chi-square results are shown. For Mann-Whitney the U and Z-score are shown. [1,] In the LMM analysis both sampling date and field code were included as random factor for moisture; [2,]ANCOVA analysis was performed for fungal biomass.

	The land use effect		**The hedgerow effect**		**The land use · hedgerow effect**	
	dfn, dfd = F	**P-value**	**dfn, dfd = F**	**P-value**	**dfn, dfd = F**	**P-value**
Moisture [1]	2, 15.0= 1.9	>0.1	1, 31.9= 7.0	***<0.05***	2, 31.9= 7.5	***<0.01***
pH (log)	2, 16.8= 15.4	***<0.001***	1, 32.4= 0.0	>0.1	2, 32.4= 0.9	>0.1
SOM	2, 17.0= 27.9	***<0.001***	1, 32.4= 5.7	***<0.05***	2, 32.4= 20.7	***<0.001***
P-Olsen (log)	2, 16.2= 29.8	***<0.001***	1, 32.0= 18.8	***<0.001***	2, 32.0= 11.8	***<0.001***
NO_x	2, 16.6= 17.1	***<0.001***	1, 31.4= 12.0	***<0.01***	2, 31.4= 12.4	***<0.001***
NH_4	2, 16.1= 5.1	***<0.05***	1, 32.2= 33.8	***<0.001***	2, 32.2= 0.8	>0.1
Total NO_x+NH_4	2, 16.3= 11.4	***0.001***	1, 31.7= 2.1	>0.1	2, 31.7= 8.6	***0.001***
C	2, 15.2= 12.9	***0.001***	1, 31.4= 19.4	***<0.001***	2, 31.4= 17.0	***<0.001***
N	2, 15.8= 26.0	***<0.001***	1, 31.6= 12.4	***0.001***	2, 31.6= 20.1	***<0.001***
N mineralisation	2, 15.8= 7.0	***<0.01***	1, 30.3= 20.5	***<0.001***	2, 30.3= 5.4	***<0.05***
C mineralisation (NP)	H (df): 0.56 (2)	>0.1	U (Z-score): 261.0 (-1.0)	>0.1	NA	NA
Bacterial biomass	2, 17.0= 50.5	***<0.001***	1, 32.0= 0.6	>0.1	2, 32.0= 30.6	***<0.001***
Actinomycetes biomass	2, 17.1= 60.5	***<0.001***	1, 32.0= 0.4	>0.1	2, 32.0= 37.7	***<0.001***
Fungal biomass (log) [2]	2, 51= 12.5	***<0.001***	1, 52= 5.7	***<0.05***	2, 51= 18.2	***<0.001***
AMF biomass (log)	2, 18.1= 70.3	***<0.001***	1, 32.3= 5.8	***<0.05***	2, 32.3= 92.2	***<0.001***
Total microbial biomass	2, 17.4= 44.8	***<0.001***	2, 32.2= 0.1	>0.1	2, 32.2= 36.0	***<0.001***
Intactness	2, 13.6= 2.9	>0.05	NA	NA	NA	NA
Height (NP)	H (df): 1.3 (2)	>0.1	NA	NA	NA	NA
Width (NP)	H (df): 7.9 (2)	***<0.05***	NA	NA	NA	NA
Bufferzone	2, 14.2= 1.2	>0.1	NA	NA	NA	NA
Shrub species	2, 13.6= 1.9	>0.1	NA	NA	NA	NA
Age (log)	2, 14.1= 1.3	>0.1	NA	NA	NA	NA
Understorey growth (NP)	H (df): 0.5 (2)	>0.1	NA	NA	NA	NA

Table S2: Output of statistical analyses of the trimmed versus untrimmed hedgerow comparison, in croplands. Linear mixed models (LMM) were performed for most properties with field code as a random factor, showed by the degrees of freedom numerator (dfn) and denumerator (dfd), F-values and P-values. Some properties were tested with non-parametric tests (Mann-Whitney), this is indicated by NP in brackets. For Mann-Whitney the U and Z-score are shown. [1.] Independent T test were performed for these properties depicted in bold, with F-value and degrees of freedom shown in brackets.

	F (dfn, dfd)	P-value
Moisture [1]	F (df): 0.1 (9)	***<0.01***
pH (log) [1]	F (df): 2.8 (9)	>0.1
SOM	1, 4.0= 1.6	>0.1
P-Olsen (log)	1, 9.0= 0.5	>0.1
NO_x	1, 3.2= 4.0	>0.1
NH_4	1, 3.9= 1.5	>0.1
Total NO_x+NH_4	1, 3.6= 3.3	>0.1
C	1, 3.9= 4.7	>0.1
N	1, 4.0= 2.8	>0.1
N mineralisation[1]	F (df): 4.2 (9)	>0.1
C mineralisation (NP)	U (Z-score): 10.0 (-0.1)	>0.1
Bacterial biomass	1, 4.0= 3.3	>0.1
Actinomycetes biomass	1, 4.0= 2.4	>0.1
Fungal biomass (log)	1, 9= 0.1	>0.1
AMF biomass (log)	1, 4.3= 0.2	>0.1
Microbial biomass	1, 4.2= 1.9	>0.1
Intactness	1, 3.6= 2.3	>0.1
Height (NP)	U (Z-score): 0.0 (-2.8)	***<0.01***
Width (NP)	U (Z-score): 10.5 (-0.8)	>0.1
Bufferzone	1, 4.1= 0.1	>0.1
Shrub species [1]	F (df): 3.6 (9)	>0.1
Age (log)	1, 4.2= 1.0	>0.1
Understorey growth (NP)	U (Z-score): 14.5 (-0.1)	>0.1

Table S3: Averages and standard errors (shown in brackets) of all hedgerow characteristics, and soil properties, categorized by land use type and hedgerow. Conservation grasslands N: 6; conservation grasslands hedgerows N: 12; production grasslands N: 6; production grasslands hedgerows N: 12; croplands N: 6; cropland hedgerows N: 11. Different letters indicate significant interactions between land use types in hedgerows or in no hedgerows (field) (P<0.05). The hedgerow effect is indicated per land use category through underlining (P<0.05).

	Cropland		**Production grassland**		**Conservation grassland**	
	Hedgerow	**No hedgerow (field)**	**Hedgerow**	**No hedgerow (field)**	**Hedgerow**	**No hedgerow (field)**
Hedgerow characteristics						
Intactness (%)	87.7 (2.62) **A**		78.0 (4.16) **AB**		89.8 (2.73) **AC**	
Age (1-3)	1.8 (0.13) **A**		1.9 (0.12) **AB**		1.6 (0.11) **AC**	
Understorey growth (1-3)	1.7 (0.14)		1.6 (0.17)		1.8 (0.18)	
Shrub species (nr/100 m)	3.7 (0.66)		5.2 (0.55)		3.0 (0.35)	
Height (m)	2.5 (0.32)		2.9 (0.21)		3.1 (0.16)	
Width (m)	0.9 (0.17) **A**		1.4 (0.16) **B**		1.4 (0.16) **B**	
Bufferzone (m)	1.4 (0.16)		1.6 (0.12)		1.8 (0.11)	
Abiotic properties						
Moisture content (%)	17.6 (1.02) **A**	21.4 (1.11)	19.4 (0.81) **AB**	19.6 (0.98)	20.6 (0.49) **B**	20.3 (0.31)
pH	6.5 (0.14) **A**	6.6 (0.28) **A**	5.7 (0.10) **B**	5.6 (0.17) **B**	5.8 (0.04) **B**	5.8 (0.08) **B**
SOM (%)	10.9 (0.35) **A**	8.1 (0.22) **A**	12.3 (0.26) **B**	12.2 (0.33) **B**	11.7 (0.35) **AB**	12.8 (0.31) **B**
P-Olsen (mg/kg)	52.7 (5.81) **A**	55.2 (6.08) **A**	37.9 (3.55) **A**	29.1 (3.83) **B**	25.9 (2.94) **B**	10.3 (0.87) **C**
NOX (mg/kg)	4025.2 (462.67)	7071.0 (987.30) **A**	4629.9 (760.67)	8864.5 (1054.28) **A**	3433.3 (263.15)	1647.4 (140.23) **B**
NH_4 (mg/kg)	1296.5 (175.59) **A**	442.9 (191.68) **A**	2653.7 (271.49) **B**	1420.2 (385.27) **B**	2044.6 (229.32) **AB**	1295.7 (282.81) **AB**
NH_4 to NO_X ratio	0.3 (0.04)	0.1 (0.04)	0.7 (0.08)	0.2 (0.04)	0.6 (0.08)	0.8 (0.20)
Total carbon (%)	4.8 (0.29) **A**	3.1 (0.18) **A**	5.6 (0.09) **B**	5.3 (0.20) **B**	5.2 (0.23) **AB**	5.4 (0.08) **B**
Total nitrogen (%)	0.4 (0.02) **A**	0.3 (0.01) **A**	0.5 (0.01) **B**	0.5 (0.02) **B**	0.5 (0.01) **AB**	0.5 (0.01) **B**
Carbon to nitrogen ratio	10.7 (0.22)	9.9 (0.38)	10.9 (0.22)	10.4 (0.20)	10.6 (0.33)	10.5 (0.32)
Biotic properties						
N mineralisation rate (N mg· kg dry $soil^{-1}$· day^{-1})	142.2 (24.05)	18.2 (15.97) **A**	155.8 (11.75)	139.9 (15.03) **B**	134.8 (9.24)	88.4 (10.44) **B**
C mineralisation rate (C mg· kg dry $soil^{-1}$· $hour^{-1}$)	0.7 (0.07)	1.0 (0.17)	0.9 (0.11)	0.7 (0.84)	0.7 (0.12)	0.8 (0.11)
Bacterial biomass (ug C ·g dry $soil^{-1}$)	28.9 (1.58) **A**	16.7 (1.15) **A**	31.9 (1.46) **AB**	35.6 (1.95) **B**	35.6 (1.32) **B**	47.1 (1.68) **C**
Actinomycetal biomass (ug C ·g dry $soil^{-1}$)	5.1 (0.26) **A**	2.7 (0.12) **A**	5.8 (0.22) **AB**	6.9 (0.31) **B**	6.2 (0.23) **B**	7.9 (0.33) **C**
Fungal biomass (ug C ·g dry $soil^{-1}$)	3.0 (0.47)	1.5 (0.26) **A**	3.3 (0.21)	1.9 (0.18) **A**	2.6 (0.10)	4.8 (0.65) **B**
AMF biomass (ug C ·g dry $soil^{-1}$)	2.9 (0.40) **A**	1.0 (0.13) **A**	1.9 (0.20) **B**	1.1 (0.12) **A**	2.1 (0.26) **AB**	18.7 (2.07) **B**
Fungal to bacterial ratio	0.10 (0.01)	0.08 (0.01)	0.10 (0.00)	0.05 (0.00)	0.07 (0.00)	0.10 (0.01)
Microbial biomass (ug C ·g dry $soil^{-1}$)	65.7 (3.88)	36.8 (2.54) **A**	69.4 (2.95)	73.9 (3.55) **B**	74.3 (2.54)	101.3 (2.39) **C**

Table S4: Averages and standard errors (shown in brackets) of all hedgerow characteristics, and soil properties, categorized by hedgerow management type in croplands (trimmed versus untrimmed hedgerows). Trimmed hedgerows N: 5, untrimmed hedgerows N: 6. The management effect is indicated through underlining (P<0.05).

	Trimmed hedgerows	**Untrimmed hedgerows**
Hedgerow characteristics		
Intactness (%)	83.5 (3.12)	91.3 (3.64)
Age (1-3)	2.0 (0.15)	1.7 (0.19)
Understorey growth (1-3)	1.7 (0.20)	1.6 (0.21)
Species (nr/100 m)	4.4 (1.25)	3.2 (0.65)
Height (m)	1.4 (0.04)	3.3 (0.23)
Width (m)	0.7 (0.04)	1.1 (0.31)
Bufferzone (m)	1.5 (0.13)	1.4 (0.28)
Abiotic properties		
Moisture content (%)	20.5 (1.07)	15.2 (0.72)
pH	6.5 (0.09)	6.4 (0.25)
SOM (%)	11.5 (0.41)	10.4 (0.47)
P-Olsen (mg/kg)	50.9 (12.39)	54.2 (4.54)
NOX (mg/kg)	5001.1 (654.61)	3212.0 (456.32)
NH4 (mg/kg)	1548.6 (276.51)	1086.4 (206.55)
NH4 to NOX ratio	0.3 (0.05)	0.3 (0.06)
Total carbon (%)	5.4 (0.36)	4.2 (0.30)
Total nitrogen (%)	0.5 (0.03)	0.4 (0.02)
Carbon to nitrogen ratio	11.3 (0.24)	10.2 (0.22)
Biotic properties		
N mineralisation rate (N mg· kg dry soil^{-1}· day^{-1})	119.5 (52.58)	161.0 (10.71)
C mineralisation rate (C mg· kg dry soil^{-1}· hour^{-1})	0.8 (0.10)	0.7 (0.09)
Bacterial biomass (ug C ·g dry soil^{-1})	32.2 (2.24)	26.1 (1.56)
Actinomycetal biomass (ug C ·g dry soil^{-1})	5.6 (0.34)	4.6 (0.31)
Fungal biomass (ug C ·g dry soil^{-1})	3.0 (0.92)	3.0 (0.48)
AMF biomass (ug C ·g dry soil^{-1})	2.6 (0.42)	3.2 (0.65)
Fungal to bacterial ratio	0.09 (0.02)	0.11 (0.02)
Microbial biomass (ug C ·g dry soil^{-1})	72.0 (6.51)	60.5 (3.85)

*Table S5: Pearson correlation analysis between soil properties. Spearman correlations were used for parameters that needed non-parametric analysis (NP). * P-values <0.05, ** P-values <0.01 (2-tailed).*

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1. Moisture	1,00															
2. pH (log_t)	0,08	1,00														
3. SOM	0,11	**-,398****	1,00													
4. P-Olsen	-0,13	**,547****	**-,309***	1,00												
5. Nox	**,351****	0,19	-0,11	0,24	1,00											
6. NH4	0,15	**-,339***	**,490****	-0,09	-0,01	1,00										
7. Total NO_x+NH_4	**,383****	0,06	0,07	0,19	**,936****	**,347****	1,00									
8. C	0,10	**-,377****	**,860****	-0,21	-0,08	**,541****	0,12	1,00								
9. N	0,12	**-,492****	**,859****	**-,339***	-0,05	**,539****	0,14	**,914****	1,00							
10. N min.	**-,363****	-0,18	**,497****	0,15	-0,20	**,295***	-0,08	**,500****	**,478****	1,00						
11. C min. (NP)	**,290***	0,10	0,02	-0,03	0,10	-0,13	0,03	-0,15	-0,11	-0,06	1,00					
12. Bacteria	0,14	**-,293***	**,699****	**-,628****	-0,19	0,20	-0,11	**,592****	**,676****	0,24	-0,02	1,00				
13. Actinomycetes	0,13	**-,371****	**,708****	**-,630****	-0,11	0,18	-0,04	**,611****	**,715****	**,273***	-0,02	**,968****	1,00			
14. Fungi (log_t)	-0,04	0,01	**,557****	**-,325***	**-,289***	,335*	-0,15	**,496****	**,480****	**,275***	0,02	**,620****	**,517****	1,00		
15. AMF (log_t)	-0,08	0,03	0,26	**-,464****	**-,511****	-0,10	**-,516****	0,11	0,21	-0,06	-0,01	**,633****	**,558****	**,543****	1,00	
16. Total biomass	0,10	-0,24	**,701****	**-,587****	-0,24	0,21	-0,15	**,592****	**,662****	**,265***	0,00	**,983****	**,938****	**,717****	**,677****	1,00

*Table S6: Pearson correlation analysis between soil- and hedgerow properties in the dataset of hedgerows only. Spearman correlations were used for parameters that needed non-parametric analysis (NP). * P-values <0.05, ** P-values <0.01 (2-tailed).*

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
1. Moisture	1,00																						
2. pH (log_t)	-0,09	1,00																					
3. SOM	**,553****	-0,27	1,00																				
4. P-Olsen	-0,19	**,610****	-0,01	1,00																			
5. Nox	**,479****	0,09	**,360***	0,19	1,00																		
6. NH4	**,516****	-0,30	**,477****	-0,09	**,460****	1,00																	
7. Total NO_x+NH_4	**,564****	-0,05	**,459****	0,11	**,939****	**,738****	1,00																
8. C	**,442****	-0,22	**,765****	0,11	**,353***	**,422***	**,432****	1,00															
9. N	**,518****	**-,396***	**,713****	-0,08	**,414***	**,490****	**,505****	**,845****	1,00														
10. N min.	-0,21	0,08	0,25	,407*	-0,18	-0,13	-0,19	0,15	0,11	1,00													
11. C min. (NP)	0,18	-0,03	0,14	-0,08	0,04	-0,06	0,00	-0,10	-0,06	0,16	1,00												
12. Bacteria	**,556****	-0,22	0,33	**-,490****	0,26	0,07	0,22	0,29	**,421***	-0,13	0,06	1,00											
13. Actinomycetes	**,553****	-0,32	**,363***	**-,528****	0,29	0,07	0,25	0,30	**,486****	-0,15	0,08	**,959****	1,00										
14. Fungi (log_t)	0,07	0,05	0,10	-0,06	0,20	0,26	0,25	0,13	0,07	0,09	0,13	0,24	0,15	1,00									
15. AMF (log_t)	-0,27	**,384***	**-,457****	-0,13	-0,23	**-,399***	-0,33	**-,515****	**-,383***	-0,06	0,22	0,08	0,00	0,25	1,00								
16. Total biomass	**,511****	-0,08	0,30	**-,388***	0,27	0,06	0,23	0,27	,358*	-0,09	0,06	**,940****	**,872****	**,489****	0,20	1,00							
17. Intactness	-0,32	0,00	**-,456****	-0,25	**-,521****	**-,613****	**-,648****	**-,529****	**-,437***	-0,14	0,04	-0,10	-0,05	-0,33	0,16	-0,16	1,00						
18. Height (NP)	-0,25	-0,04	0,05	0,05	-0,30	-0,03	-0,25	-0,11	-0,13	0,31	0,01	0,02	0,02	0,14	-0,02	0,06	0,12	1,00					
19. Width (NP)	-0,05	**-,357***	0,32	-0,31	-0,29	0,18	-0,21	0,23	0,23	0,11	0,04	0,12	0,16	0,15	0,02	0,16	-0,10	**,449****	1,00				
20. Buffer zone	0,17	-0,17	0,01	**-,355***	-0,25	0,16	-0,15	0,07	0,12	-0,07	-0,21	0,28	0,27	0,14	0,11	0,31	-0,05	0,01	**,565****	1,00			
21. Species	0,04	0,10	0,26	0,30	0,03	0,17	0,09	0,19	0,07	0,33	0,01	-0,19	-0,24	-0,08	-0,14	-0,15	**-,421***	-0,24	-0,01	0,05	1,00		
22. Age (log_t)	0,22	0,03	**,471****	0,23	0,32	0,23	0,34	**,370***	**,379***	0,20	0,16	0,19	0,21	**,385***	-0,05	0,30	**-,388***	0,01	0,14	-0,23	0,06	1,00	
23. Understorey (NP)	0,09	0,02	0,09	-0,08	0,04	0,14	0,05	-0,03	-0,01	**,400***	-0,01	0,20	0,11	0,08	0,12	0,26	-0,28	0,07	0,12	**,391***	**,404***	0,15	1,00

Table S7: The results from the combined PCA and RDA analysis with different dependent and explanatory datasets. The adjusted explained variation is based on the PCA analysis.

	Model 1	**Model 2**	**Model 3A**	**Model 3B**	**Model 4**
Selection	NA	hedges	NA	NA	hedges
Dependent dataset	Soil properties	Soil properties	Soil biota	Soil biota	Soil biota
Explanatory dataset	L*H	L*H & hedgerow	Abiotic	L*H & Abiotic	L*H & hedgerow & abiotic
Total variation	79.500.000	49.500.000	37.100.000	37.100.000	23.100.000
Explained variation (Adj) (%)	48.8	28.7	54.1	63.9	34.4
Variable 1 (V1) (P<0.05)	CF	CWH	SOM	SOM	Moisture
V1: Explained variation (%)	22.9	15.5	31.1	31.1	17.0
Variable 2 (V2) (P<0.05)	NF	Intactness	P-Olsen	NF	P-Olsen
V2: Explained variation (%)	14.6	9.5	11.8	18.3	7.9
Variable 3 (V3) (P<0.05)	CWH	CCH	log-pH	CF	log-pH
V3: Explained variation (%)	7.5	5.7	6.7	7.5	7.6
Variable 4 (V4) (P<0.05)	PF	NA	Moisture	P-Olsen	log-age
V4: Explained variation (%)	5.0	NA	5.3	3.6	5.8
Variable 5 (V5) (P<0.05)	CCH	NA	NOx	log-pH	NA
V5: Explained variation (%)	2.7	NA	2.9	3.8	NA
Variable 6 (V6) (P<0.05)	PWH	NA	NA	PWH	NA
V6: Explained variation (%)	2.0	NA	NA	2.0	NA