Nutrient Response Efficiencies, Leaching Losses and Soil-N Cycling in Temperate Grassland Agroforestry and Open Grassland Management Systems

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Summary

In recent years, there is an increasing interest in innovative agricultural systems as an alternative to open grassland systems in order to minimize the detrimental effects of intensive production systems on the environment such as nutrient leaching to ground and surface waters. One possible solution could be agroforestry, i.e. the implementation of trees into grassland in the form of alternating rows, also known as alley cropping system. By fostering ecological interactions between woody and non-woody components, agroforestry systems can minimize the detrimental effects of intensive production mentioned above. These systems are therefore seen as more sustainable and environmentally friendly production systems than intensive production systems or monocultures. At present, however, regarding temperate agroforestry, it is unknown whether this particular agroforestry system is a sustainable and environmentally friendly alternative to open grassland in terms of nutrient and water use. The overall aim of this thesis therefore was to test whether grassland agroforestry (alley cropping of grasses and fast growing trees) is a sustainable alternative to open grassland by investigating the index of **nutrient response efficiency**, **nutrient leaching losses** and the **soil-N cycle**. Agroforestry and open grassland systems were investigated on three soil types (Histosol, Anthrosol and Cambisol soils) in central Germany in 2016 and 2017. Measurements in the agroforestry systems were conducted in the tree rows and at various distances to the tree rows within the grass rows in four (Histosol and Anthrosol soils) or three (Cambisol soil) replicate plots.

The first study aimed to assess differences in **nutrient response efficiency** (NRE, ratio of biomass production to soil available nutrient) and plant-available nutrients between grassland agroforestry and open grassland. Plant available N and P were measured several times during the vegetation period using the buried bag method for N and a resin- and bicarbonate-extraction for P. The cations Ca, K and Mg were determined once. Biomass production was either measured (grass) or calculated by using allometric functions (trees). Plant-available N, P, macronutrients and NREs were generally comparable between agroforestry and open grassland, suggesting no net effect of competition or complementarity for nutrients between trees and grasses. One exception were the marginally lower Ca and Mg response efficiencies in agroforestry compared to open grassland in the Cambisol soil. This was due to the narrow grass rows (9-m wide), which showed lower biomass of grasses at 1 m from the tree row, possibly due to the trees' shading. In 2017, when tree production was higher in the second year after tree harvest, agroforestry had higher P and K response efficiencies than open grassland for Histosol and Anthro-

sol soils. It is therefore concluded that alley cropping agroforestry can be a sustainable alternative to open grassland without sacrificing NRE and soil nutrient availability, particularly in systems with wide grass rows (48-m wide) and when trees are getting older.

The second study aimed to quantify **nutrient leaching losses** in temperate alley cropping systems of alternating rows of fast growing willows and grassland. Nutrient leaching losses were calculated by multiplying monthly measured nutrient concentrations in soil water at 0.6 m depth from each sampling point with modelled monthly leaching fluxes. For all soil types tree rows displayed high interception rates resulting in water drainage fluxes that were considerably lower compared to the grass rows. At all three sites NO_3^-, phosphate and base cation (Ca, K, Mg) leaching losses were highly variable throughout the study year and did not differ between tree rows and grass rows because of considerable temporal variability. However, looking into ratios of Na/nutrient showed that trees positively influenced nutrient losses by preferential nutrient uptake and possibly due to increased microbial processes such as denitrification under tree rows. The study thus provides evidence that fast growing trees in temperate grassland alley cropping systems can contribute to reduction of leaching losses and to better ground water quality.

The third study aimed to assess differences in **gross rates of soil-N cycling** between agroforestry and open grassland, and their controlling factors. To determine gross rates of soil-N-cycling processes (i.e. gross N mineralization, gross nitrification, N immobilization and dissimilatory NO_3^- reduction to ammonium) the ^{15}N pool dilution technique was used on intact soil cores. There were no significant differences in gross rates of mineral N production (mineralization and nitrification) between sampling locations in grassland agroforestry and open grasslands within soil types. Management (grassland agroforestry vs open grassland) or vegetation type (tree or grass) did not affect soil-N cycling, thus opposing the hypothesis that gross rates of soil-N cycling are higher in the tree rows than in the grass rows or open grassland. Instead, N-cycling rates and microbial biomass were rather affected by soil types. Grassland agroforestry can thus be equally efficient in terms of soil N availability and soil-N cycling compared to open grassland and can be a sustainable alternative management system.

This thesis overall shows that temperate grassland agroforestry can be a sustainable alternative management system to open grassland in terms of NRE, nutrient leaching and by sustaining an active N-cycling. However, multi-year evaluations, both of the economic benefit and the ecological functions, are needed in order to quantify long-term trends, which could then provide a robust basis for inclusion of agroforestry into a broader framework of policy.

Zusammenfassung

In den letzten Jahren besteht ein zunehmendes Interesse an innovativen landwirtschaftlichen Systemen, um die nachteiligen Auswirkungen intensiver Produktionssysteme, wie z. Nährstoffauswaschung in Grund- und Oberflächengewässer, auf die Umwelt zu minimieren. Eine mögliche Lösung in Grünlandsystemen könnte Agroforstwirtschaft sein, die Integration von Bäumen z.B. in Reihen. Durch ökologische Wechselwirkungen zwischen den holzigen und nicht holzigen Pflanzen können Agroforstsysteme die oben genannten nachteiligen Auswirkungen einer intensiven Produktion minimieren. Agroforstsysteme gelten daher generell als nachhaltigere und umweltfreundlichere Produktionssysteme im Vergleich zu Monokulturen. In den gemäßigten Zonen ist derzeit jedoch nicht bekannt, ob dieses spezielle Agroforstsystem mit abwechselnden Reihen von Grünland und Bäumen (alley-cropping) eine nachhaltige und umweltfreundliche Alternative zu offenem Grünland im Hinblick auf die Nährstoff- und Wassernutzung darstellt. Das übergeordnete Ziel dieser Dissertation war es daher zu untersuchen, ob Grünlandagroforst (abwechselnde Reihen von Grünland und schnell wachsenden Bäumen) eine nachhaltige Alternative zu offenem Grünland darstellt. Dafür wurden die Indices **nutrient response efficiency (NRE)**, die **Nährstoffverluste durch Auswaschung** und der **N-Kreislauf** im Boden untersucht. In den Jahren 2016 und 2017 wurden in Mitteldeutschland offene und Agroforstgrünlandsysteme auf drei Bodentypen (Histosol, Anthrosol und Cambisol) untersucht. Messungen in den Agroforstsystemen wurden in den Baumreihen und in verschiedenen Abständen zu den Baumreihen innerhalb der Grasreihen in vier (Histosol- und Anthrosol-Böden) oder drei (Cambisol-Böden) Wiederholungsparzellen durchgeführt.

Die erste Studie hatte zum Ziel Unterschiede in der **nutrient response efficiency** (NRE, Verhältnis von Biomasseproduktion zu pflanzenverfügbaren Nährstoffen) und den pflanzenverfügbaren Nährstoffen zwischen Grünlandagroforst und offenem Grünland zu bewerten. Pflanzenverfügbares N und P wurden während der Vegetationsperiode mehrmals mit der Buried-Bag-Methode für N und einer Resin- und Bicarbonatextraktion für P gemessen. Die Kationen Ca, K und Mg wurden einmal gemessen. Die Biomasseproduktion wurde entweder gemessen (Gras) oder mittels allometrischer Funktionen (Bäume) berechnet. Pflanzenverfügbare N, P, Makronährstoffe und NREs waren im Allgemeinen zwischen Grünlandagroforst und offenem Grünland vergleichbar, was darauf hindeutet, dass es weder Konkurrenz noch Komplementarität zwischen Bäumen und Gräsern um Nährstoffe gab. Eine Ausnahme bildeten die geringfügig niedrigeren Ca- und Mg-NREs im Grünlandagroforst im Vergleich zu offenem Grünland im

Cambisol. Dies war auf die schmalen Grasreihen (9 m breit) zurückzuführen, die in 1 m Entfernung von der Baumreihe eine geringere Biomasse der Gräser aufwiesen, möglicherweise aufgrund der Beschattung der Bäume. Im Jahr 2017, als die Baumproduktion im zweiten Jahr nach der Baumernte höher war, wies Grünlandagroforst für Histosol- und Anthrosol höhere P- und K-nutrient response efficiencies als offenes Grünland auf. Es wird daher der Schluss gezogen, dass Agroforstwirtschaft eine nachhaltige Alternative zu offenem Grünland darstellen kann, ohne dass die Verfügbarkeit von Bodennährstoffen oder die NREs beeinträchtigt wird. Dies ist insbesondere in Systemen mit breiten Grasreihen (48 m breit) der Fall und wenn die Bäume älter werden.

Die zweite Studie sollte die **Verluste von Nährstoffen durch Auswaschung** in Grünlandagroforst mit abwechselnden Reihen von schnell wachsenden Weiden und Grünland quantifizieren. Die Nährstoffverluste wurden berechnet, indem die monatlich gemessenen Nährstoffkonzentrationen im Bodenwasser in 0,6 m Tiefe von jedem Probenahmepunkt mit modellierten monatlichen Abflüssen multipliziert wurden. Bei allen Bodentypen wiesen die Baumreihen eine hohe Interzeption auf, was zu Wasserverlusten führte, die im Vergleich zu den Grasreihen erheblich niedriger waren. An allen drei Standorten waren die Auswaschungsverluste von NO_3^-, Phosphat und Kationen (Ca, K, Mg) während des gesamten Untersuchungsjahres sehr variabel und unterschieden sich aufgrund der erheblichen zeitlichen Variabilität nicht zwischen Baum- und Grasreihen. Die Untersuchung der Na/Nährstoff-Verhältnisse ergab jedoch, dass die Bäume Nährstoffverluste durch die bevorzugte Nährstoffaufnahme und möglicherweise durch vermehrte mikrobielle Prozesse wie die Denitrifikation unter Baumreihen positiv beeinflussten. Die Studie liefert somit Belege dafür, dass schnell wachsende Bäume zur Verringerung der Auswaschungsverluste auf Grünland und zur Verbesserung der Grundwasserqualität beitragen können.

Die dritte Studie zielte darauf ab, Unterschiede im **N-Kreislauf im Boden** zwischen Grünlandagroforst und offenem Grünland sowie dessen Einflussfaktoren zu bewerten. Zur Bestimmung der Bruttoraten von Boden-N-Kreislaufprozessen (d. H. Brutto-N-Mineralisierung, Brutto-Nitrifizierung, N-Immobilisierung und dissimilatorische Nitritreduktion zu Ammonium) wurde die ^{15}N-Poolverdünnungstechnik verwendet. Es gab keine signifikanten Unterschiede bei den Bruttoraten der N-Produktion (Mineralisierung und Nitrifizierung) zwischen den Probenahmestellen in Agroforst und offenen Grünland innerhalb der drei Bodentypen. Die Bewirtschaftung (Grünlandagroforst im Vergleich zu offenem Grünland) oder der Vegetationstyp (Baum oder Gras) wirkten sich nicht auf den Boden-N-Kreislauf aus, was der Hypothese widerspricht, dass die Bruttoraten des Boden-N-Kreislaufs in den Baumreihen höher sind als in den Grasreihen oder im offenen Grünland. Stattdessen wurden der N-Kreislauf und die mikrobielle Biomasse eher von den Bodentypen beeinflusst. Grünlandagroforst kann daher in Bezug auf die Verfügbarkeit von N und den gesamten N-Kreislauf im Boden im Vergleich zu offenem Grünland gleichermaßen effizient sein und ein nachhaltiges alternatives Managementsystem darstellen.

Insgesamt zeigt diese Arbeit, dass Agroforstwirtschaft ein nachhaltiges alternatives Managementsystem zu Grünland in Bezug auf NRE, Nährstoffauswaschung und die Aufrechterhaltung eines aktiven N-Kreislaufs sein kann. Es sind jedoch mehrjährige Bewertungen sowohl des wirtschaftlichen Nutzens als auch der ökologischen Funktionen erforderlich, um langfristige Trends zu quantifizieren, die dann eine solide Grundlage für die Einbeziehung der Agroforstwirtschaft in einen breiteren politischen Rahmen bilden könnten.

1 General introduction

1.1 The land-use trilemma

Feeding the growing world population necessitates intensive agricultural production. While modern agriculture is very productive it strongly depends on high fertilizer application rates which can cause detrimental environmental effects (Tilman et al. 2002). Excess nutrients lost from agricultural land by leaching to surface or groundwater, or by gaseous emissions to the atmosphere, can cause severe problems for the environment (e.g. Hoeft et al. 2014, Abalos et al. 2018). High leaching losses of nutrients, especially nitrate (NO_3^-), can cause eutrophication of surface waters, a loss in biodiversity and also pose a health risk for humans (e.g. Di and Cameron 2002, Isbell et al. 2013, WHO 2017). Additionally, these losses may entail economic consequences e.g. rising costs for water treatment or difficulty of reliably providing high-quality drinking water (Price and Heberling 2018). Therefore, there is a strong need for new methods in agriculture to secure food production while maintaining environmental quality (Matson et al. 1997). So far, intensification has mainly concentrated on high usage of fertilizer, pesticide and water as well as new crop strains, but not on ecological interactions within agricultural systems. These ecological interactions, however, are increasingly necessary since conventional agricultural management systems while being productive and profitable often fail in efficiently using resources such as soil nutrients (Tilman et al. 2002). Fostering ecological benefits, therefore, is crucial in order to obtain highly productive agriculture with less negative environmental impacts (Robertson and Swinton 2005). Enhancing this goal could also be a significant component to mitigate the “land-use trilemma”: The need to balance food-security, biofuel production and reduction of greenhouse-gases caused by an increasing demand both for food and the land to produce it as well as the ongoing climate change (Tilman et al. 2009).

Grassland is an important agricultural production system. This type of land-use is not only essential for agricultural production, but also for sustaining ecological functions such as habitat for biological activity, carbon sequestration, filtering and storage of water, nutrient storage and recycling. These ecological functions are non-monetary benefits both for farmers and society. Grassland worldwide currently covers 70% of the agricultural land (FAO 2018). In Germany, grassland takes up about one third (4.7 Mio ha) of the utilized agricultural area (Destatis 2016). In general, less negative environmental effects are reported from agriculturally used grasslands than from cropland. Grasslands are typically either used intensively (e.g. regular fertilization, artificial drainage, high animal stocking rates or frequent cutting), or, if they are e.g. located at sites with low productivity, are managed extensively (Isselstein et al. 2005, Gilhaus et al. 2017). However, over the last decades, grasslands in Europe have suffered from agricultural intensification e.g. increased fertilization and cutting rate (Isselstein et al. 2005), which makes them significant sources for leaching losses and trace gas emissions (Di and Cameron 2002, Flechard et al. 2007). Grassland farming therefore is under increasing pressure for improved management strategies, especially approaches for improving the efficiency of the soil-nitrogen (N) cycling, such that negative effects of high fertilizer use can be minimized (e.g. Jones et al. 2005, Cameron et al. 2013, Hoeft et al. 2014). While there are many studies on leaching from cropland, leaching from grassland is often overlooked as it is considered to be lower in comparison to cropland (Di and Cameron 2002). Effective methods to reduce leaching losses, are increasingly needed, however, from this land-use system as well. This has become specifically urgent in 2018, when the European court of justice sued Germany for not taking strong enough actions for protection of waters against pollution caused by NO_3^- leaching from agricultural sources (EU 2018). Since grasslands offer a wide range of ecosystem services and are a habitat for many species (Wilson et al. 2012, Martin et al. 2014), it is crucial to assess their soil-N cycling, which can indicate whether soil N availability is sufficient or in excess, which could lead to negative environmental effects. The intensified use of grasslands applying innovative agricultural methods could be one aspect to solve the “land-use trilemma” mentioned above.

One component of the German strategy to increase the production and use of renewable energy until 2020 is the use of fast growing trees to produce woody biomass (BMELV 2009). This is necessary since the European Union (EU) has claimed the initiative to produce 20 % of primary energy supply by renewable resources until 2020 (EU 2009). Consequently, the importance of the energetic use of wood in Germany has grown (e.g. Mantau 2012). However, this strong increase in demand resulted in a lack of required biomass for the German energy and material related wood market, the “wood gap” (Thrän et al. 2009). Since fertile land is limited, interest in agricultural management systems that allow several purposes at once (e.g. provision of food, energy, and ecosystem services) to avoid land-use conflicts and solve the “land-use trilemma”, has strongly risen. One such agricultural management system could be the implementation of trees onto existing grassland as agroforestry systems (Tilman et al. 2009). This management system, potentially stimulating ecological functions, could be a possible alternative to the intensive production systems should it be able to provide ecosystems services, e.g. erosion control, soil fertility and organic matter conservation, without sacrificing productivity (Torralba et al. 2016, Kay et al. 2019).

1.2 Agroforestry as a sustainable alternative land-use system

Agroforestry is defined as "the practice of deliberately integrating woody vegetation (trees or shrubs) with crop and / or animal systems to benefit from resulting ecological and economic interactions" (Burgess and Rosati 2018). An important ecosystem service of agroforestry systems is the provision of biomass of woody and non-woody components (food / fodder) at the same time. Whereas agroforestry systems in the tropics are well known management practices, they have vanished in temperate areas over the last century due to mechanization and intensification of agricultural production (Nerlich et al. 2013). Consequently, only little research has been conducted on temperate agroforestry systems in recent years. Agroforestry systems in temperate areas include e.g. hedges for windbreaks, grazed or intercropped orchards, silvopastures and riparian buffer strips (Mosquera-Losada et al. 2012). The current need for management systems that include environmental enhancement could lead to a "renaissance of agroforestry" (Torralba et al. 2016). Recently, especially alley cropping with alternating rows of fast-growing trees for bioenergy production and rows of grass- or cropland is seen as a successful management strategy (Tsonkova et al. 2012, Langenberg and Theuvsen 2018). The tree rows are harvested in short rotations of three to six years using fast-growing tree species, such as willow or poplar, which resprout after cutting. With this management strategy, farmers can diversify the provision of market goods, while at the same time maintaining a high degree of mechanization (Tsonkova et al. 2012). Furthermore, these alley cropping systems can be developed on marginal land and / or intensively cultivated unfertile land with high environmental risks (e.g. wind erosion). Here, the ecological and economic benefits from alley cropping could be high and the opportunity costs are relatively low (Böhm et al. 2014).

Since agroforestry systems are composed of woody and non-woody components differing in e.g. harvest cycles (one harvest every several years vs. several harvests per year), research acquisition (deeper vs. lower rooting) and nutrient export (nutrients taken up by trees keep cycling in the system through decomposing tree litter vs. nutrients taken up by grasses mostly exported with harvested biomass), one can expect differences in the nutrient efficiencies of these innovative management systems compared to traditional open grassland systems. However, at present, no study has reported whether grassland agroforestry renders beneficiary, neutral or negative effects versus open grasslands in terms of resource use (i.e. soil nutrients). Such field-based data are crucial for developing policies geared towards reducing the negative impacts from grassland (e.g. the European Union's Common Agricultural Policy).

In this thesis therefore, two grassland management systems in Lower Saxony, Germany, were investigated: grassland agroforestry with the alley cropping agroforestry systems having alternating rows of fast growing trees for bioenergy production and grass rows for fodder production and open grassland (i.e. only grasses).

There are several suitable ecological methods to assess whether agroforestry is an effective alternative to conventional agriculture. One method to evaluate the effectiveness of management systems is to use an integrative metric of productivity in relation to plant-available nutrients in the soil, i.e. **nutrient response efficiency (NRE)**. This index has been used to evaluate ecosystem functioning in temperate grasslands (Keuter et al. 2013) as well as both, tropical and temperate forest ecosystems (Hiremath and Ewel 2001, Schmidt et al. 2015). The index is calculated as productivity per unit of plant-available nutrient (Bridgham et al. 1995). The relationship of NRE with plant-available nutrients is described by a unimodal curve, with the lowest NRE values at the minimum level of plant-available nutrient, the highest NRE values at the optimum nutrient level, and decreasing NRE values beyond the optimum nutrient level towards nutrient saturation (Fig A1, Pastor and Bridgham 1999).

The central hypothesis of agroforestry is that the integration of trees into agricultural systems results in a more efficient acquisition of resources (e.g. soil nutrients, water) if trees acquire nutrients from deeper soil layers beyond the reach of non-woody components (Canell et al. 1996). It has been confirmed that trees take up and distribute water and nutrients from deeper layers via hydraulic lift (Burgess et al. 2001). In a six-year-old grassland agroforestry system with poplar trees and grassland, trees took up cations from deeper soil layers (Mosquera-Losada et al. 2011); these cations were then incorporated in the silvopastoral system through the decomposing tree litter. The trees were thus acting as a nutrient pump. In contrast, on fertile croplands in Belgium nutrient availability did not increase in young alley-cropping systems (< 5 years), but only close to older trees (15–47 years) in field boundaries (Pardon et al. 2017). Earlier studies have shown that several mechanisms contribute to reduced nutrient leaching if trees or shrubs are integrated into cropland. Deep tree roots can act as a “safety-net” by taking up nutrients below the reach of crop roots, or trees can assimilate nutrients at times when nutrient demand from crops is low (e.g. Jose et al. 2004, Bergeron et al. 2011). These effects are expected to be most effective close to the trees and decrease at increasing distance to the trees since the density of tree roots and the trees’ shade are decreasing with distance from the trees (e.g. Pardon et al. 2017). In contrast to cropland, grassland has a permanent root system and at present only few studies have investigated whether integration of trees or shrubs in grassland has similar positive effects as those described for cropland. Riparian buffers of switchgrass (*Panicum virgatum*) and woody components had a higher efficiency in removing nutrients compared to pure switchgrass buffers (Lee et al. 2003). Furthermore, lower NO_3^--concentration in leachate was found under silvopasture compared to conventional pasture or a hardwood forest in West Virginia, US. This was probably due to more efficient NO_3^- uptake in the silvopastoral system, higher losses in the pure forest and due to a high return of N with leaf fall in autumn (Boyer and Neel 2010). In this thesis the influence of trees and grasses on **nutrient concentrations in soil water, nutrient leaching losses and drainage fluxes** was therefore investigated as an index for environmental quality.

An efficient N-cycle is important for a functioning plant-soil system with a high production and only little N-losses. Little is known on how soil-N cycling changes between grassland agroforestry and open grasslands in temperate areas. In particular, there is a lack of quantitative measurements on the mineral N production (i.e. gross N mineralization and nitrification rates) and retention processes (i.e. N immobilization and dissimilatory NO_3^- reduction to ammonium, NH_4^+, [DNRA]) in such grassland alley cropping agroforestry and open grasslands. Changes in soil-N cycling with management are often related to the feedbacks between the size of the soil microbial community and quantity / quality of actively cycling organic matter (Corre et al. 2003). On assuming that the trend in belowground net primary production (BNPP) in a grassland alley cropping system is similar to aboveground net primary production (ANPP), tree rows will have more organic material input from litter production than the grass rows in grassland agroforestry systems (Göbel et al. unpublished data). As to organic matter quality, indicated by C:N ratio, grassland soils generally have lower C:N ratios than soils under trees due to recalcitrant lignin compounds derived from tree litter (e.g. Booth et al. 2005). However, trees support a greater bacterial abundance than pure grasslands and promote a higher fungi to bacteria ratio than crop or grass rows of temperate alley cropping systems (Banerjee et al. 2016, Beuschel et al. 2018, Beule et al. 2019). Additionally, microbial biomass C contents were higher in woodlands and short rotation forests than in the original grasslands these forests initially developed on (Chen et al. 2003, Liao and Boutton 2008). Management effects on soil microbial biomass and, in turn, on soil-N cycling are ultimately controlled by indicators of soil fertility (e.g. soil C:N, biodegradable organic C, pH, effective cation exchange capacity [ECEC] and base saturation). In a temperate grassland lower C:N ratio and higher biodegradable organic C in the drained, lower landscape position showed higher microbial biomass and gross N mineralization rates than in the upper landscape position (Corre et al. 2002). The same pattern was seen with higher gross N mineralization rates in grassland under an oak canopy with a higher inorganic N pool and total C content than in an open grassland with lower inorganic N pool at the same study site (Davidson et al. 1990). Reduction of acid input (= pH improvement) into a temperate spruce forest led to slightly increased N mineralization rates (Corre and Lamersdorf 2004). Thus, trees in agroforestry systems may affect the rates of soil-N cycling through their influence on substrate quantity and quality which, in turn, affect microbial biomass size and composition. In this thesis **gross rates of soil-N cycling** were therefore used as an index for soil N availability to compare the two different management systems (e.g. Hoeft et al. 2014, Allen et al. 2015).

1.3 Objectives and hypotheses

The aim of this thesis was to test whether grassland agroforestry is a sustainable alternative to open grassland by investigating the index of nutrient response efficiency, nutrient leaching losses and the soil-N cycle. The following three studies were conducted:

STUDY I: CAN TEMPERATE GRASSLAND AGROFORESTRY BE A SUSTAINABLE ALTERNATIVE TO OPEN GRASSLAND IN TERMS OF SOIL NUTRIENT AVAILABILITY AND NUTRIENT RESPONSE EFFICIENCY?

The study aimed to assess differences in NRE and plant-available nutrients between temperate grassland agroforestry (i.e. alley cropping of fast-growing willow trees and grassland) and open grassland (i.e. grassland without trees).

Hypothesis:

1) Based on an increase in productivity in the agroforestry system due to the trees, nutrient response efficiency will be higher in grassland agroforestry compared to open grassland.

STUDY II: DO FAST GROWING TREES REDUCE NUTRIENT CONCENTRATION IN SOIL WATER AND LEACHING LOSSES IN TEMPERATE GRASSLAND AGROFORESTRY?

The objective was to evaluate the impact of fast growing trees in grassland agroforestry systems on nutrient leaching losses by comparing the different components of this agroforestry system (i.e. tree rows and grass rows).

Hypotheses:

1) Nutrient leaching losses will be smaller in the tree rows than in the grass rows of the agroforestry systems, and
2) in the grass rows, nutrient losses will increase with distance from the tree rows.

STUDY III: HOW DO GROSS RATES OF SOIL-N CYCLING IN TEMPERATE GRASSLAND AGROFORESTRY AND OPEN GRASSLAND DIFFER?

The aims were to assess differences in gross rates of soil-N cycling between grassland agroforestry and open grassland, and their controlling factors.

Hypothesis:

1) If the short rotation trees increase microbial biomass size as a consequence of increase in organic matter input from litter fall and root turnover, gross rates of soil-N cycling will be higher in the tree rows than in the grass rows or open grassland.

2 Material and methods

2.1 The SIGNAL project

This thesis was carried out within the first phase (2015‒2018) of the interdisciplinary project SIGNAL (Sustainable intensification of agriculture through agroforestry). SIGNAL is part of the BONARES initiative “Soil as a sustainable resource” funded by the German ministry of education and research (BMBF). The project sites were situated in central Germany at four cropland agroforestry sites (Reiffenhausen, Wendhausen, Forst, Dornburg) and two grassland agroforestry sites (Reiffenhausen, Mariensee; Fig 1). Each site consisted of an alley cropping agroforestry system (i.e. alternating rows of fast growing trees for bioenergy production and rows of cropland or grassland) with an adjacent open system to compare the two management systems. Agroforestry and open management systems were cultivated in the same way regarding e.g. fertilization, harvesting, soil cultivation, and plant protection, the presence of trees in the area managed as agroforestry systems being the only difference.

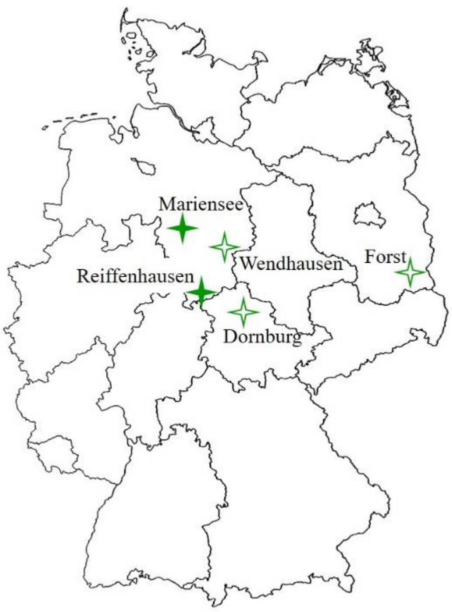


Fig 1 Overview of the study sites in the SIGNAL project. The grassland agroforestry sites are situated in Mariensee and Reiffenhausen.

2.2 Study sites

Work for this thesis was conducted at the two study sites (Reiffenhausen, Mariensee) with grassland agroforestry systems, used for fodder and bioenergy production in lower Saxony, Germany (Fig 1). These are two of the very few established grassland alley cropping systems in Germany and represent a range of plant-available nutrients in the soil. Site characteristics and management practices of both study sites are described in Table 1. The grassland site near

Mariensee (52° 33’ 49” N, 9° 28’ 9” E, 42 m above sea level, asl) has two soil types, Histosol soil (even if the peat horizon does not extend to 0.4-m depth in all places) and Anthrosol soil; the grassland site close to Reiffenhausen (51° 23’ 52” N, 9° 59’ 29” E, 323 m asl) has Eutric Cambisol to Eutric Stagnic Cambisol soil. In the following, the sites will be referred to according to their soil types. During the study period (April 2016–April 2017) the site with Histosol and Anthrosol soils received 583 mm precipitation and had a mean annual temperature of 10.3 °C; the site with Cambisol soil received 544 mm precipitation and had a mean annual temperature of 9.8 °C. During the study period, both sites had a higher mean annual temperature but lower precipitation than the 30 year average (Histosol and Anthrosol soils: 661 ± 20 mm, 8.7 ± 0.3 °C, mean ± standard error, climate station at Hanover of the German Meteorological Service, 1981−2010; Cambisol soil: 651 ± 24 mm, 9.2 ± 0.1 °C, climate station at Goettingen of the German Meteorological Service, 1981−2010).

Table 1 Site characteristics and management practices of the investigated agroforestry (AF) and adjacent open grasslands on three soil types in central Germany.

Soil type	Management system		Establishment	Harvest	Plot size	Row width	Species	Fertilization
Histosol/Anthrosol soil	AF	tree	2008	1st harvest Jan. 2016	10 x 26.5 m	11.4 m	*Salix schwerinii* x *S. viminalis*	none
		grass	before at least 1990, reseeded 2008	cut (June 2016 and 2017) and mulched (Oct. 2016 and Oct. 2017)		48 m	*Lolium perenne*, *Festuca pratensis*, *Phleum pratense*, *Poa pratensis*	Nov. 2015 digestate (50 kg N ha^{-1}, 8.5 kg P ha^{-1}, 46 kg K ha^{-1}, 4.5 kg Mg ha^{-1}, 12 kg Ca ha^{-1})
	Open		before at least 1990		10 x 10 m	-		
Cambisol soil	AF	tree	2011	1st harvest Jan. 2015	6.5 x 6.5 m	7.5 m	(*S. schwerinii* x *S. viminalis*) x *S. viminalis*	none
		grass		cut (June, July, Sept. 2016; Mai, July, Aug., Oct. 2017)		9 m	*Lolium perenne*, *Trifolium repens*	April 2017 (250 kg PK ha^{-1}, 550 kg Mg ha^{-1})
	Open		2014		6 x 6 m	-		

2.3 Experimental design

To investigate the aims of this thesis, three (Histosol and Anthrosol soils) or four (Cambisol soil) replicate plots were selected per soil type (Figs 2 and 3).

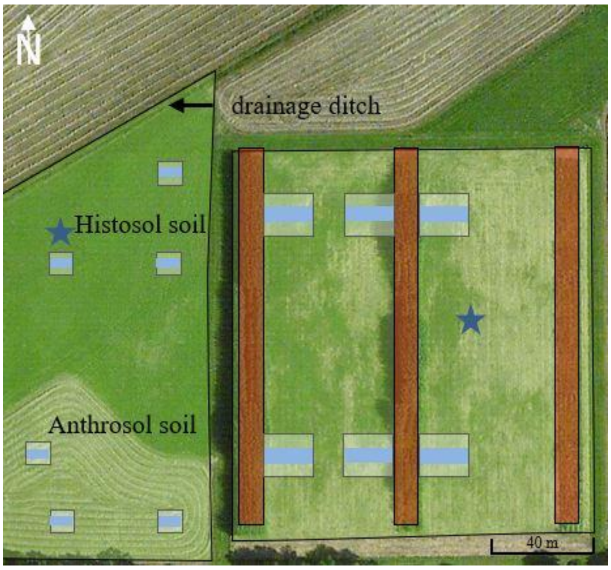


Fig 2 Experimental design at the site Mariensee, Lower Saxony, Germany. This site consists of two soil types (Histosol soil in the northern part, Anthrosol soil in the southern part), therefore three replicate plots per soil type and management system were established.

(adapted from M. Schmidt, http://geoviewer.bgr.de/mapapps/resources/apps/geoviewer/index.html?lang=de)

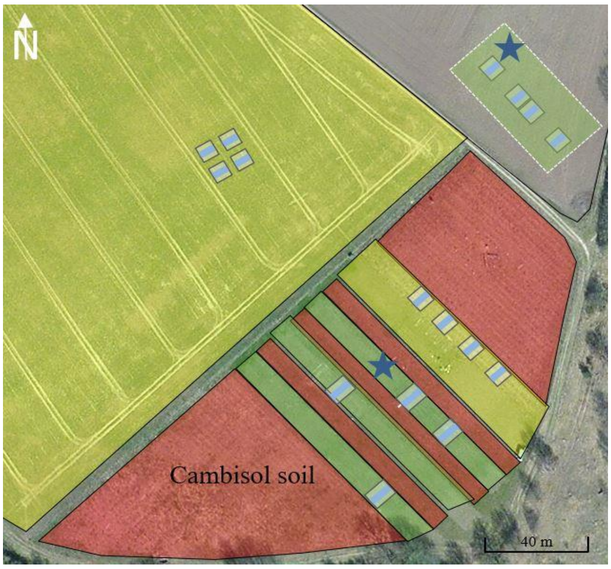
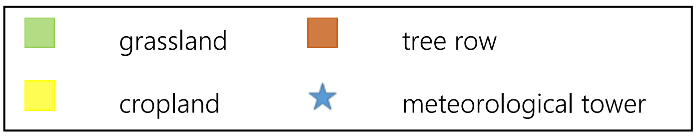


Fig 3 Experimental design at the site Reiffenhausen, Lower Saxony, Germany, with four replicate plots per management system.

(adapted from M. Schmidt, http://geoviewer.bgr.de/mapapps/resources/apps/geoviewer/index.html?lang=de)



Within agroforestry, sampling locations per replicate plot were located within the tree rows and at 1-m, 4-m (both sites) and 7-m distance (only Histosol and Anthrosol soils) to the tree rows (Fig 4 a and b). In open grassland sampling locations were located in the middle of the plots (Fig 4 c and d).

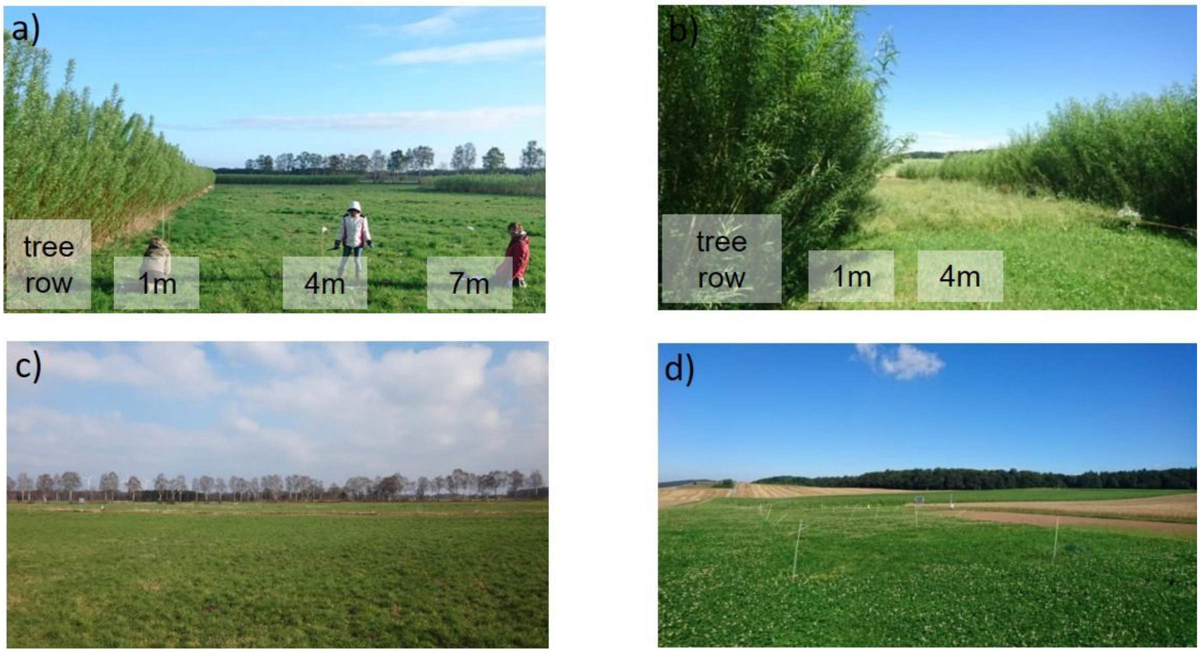


Fig 4 Sampling design in grassland agroforestry and open grassland management systems for Histosol and Anthrosol soils (a and c) and Cambisol soil (b and d).

In the experimental design the inherent assumption was that the initial soil conditions between the two management systems at each site were similar prior to the establishment of the agroforestry systems. To test this assumption, a land-use-independent soil characteristic (soil texture) was used as a surrogate variable to infer whether there were differences in the initial soil characteristics between the grassland agroforestry and open grassland systems within each soil type (e.g. Allen et al. 2015, Corre et al. 2007). No significant differences in soil texture between these systems at any site were detected (Table 2). Hence, observed differences in e.g. NRE and soil nutrient availability can be attributed to the differences in management.

During field work, it was found that ground water fluxes at the site with Histosol and Anthrosol soils strongly differed between grassland agroforestry and open grassland due to a drainage ditch close to the open grassland (Fig 2). Hence, the assumptions that the management systems only differ in the presences or absence of trees was not given at this site and therefore study two (leaching losses) solely concentrated on differences in leaching losses within grassland agroforestry. The other studies however were not affected, since soil characteristics, nutrient availability and biomass production was similar between the management systems (see results). On Cambisol soil plant composition of open grassland and the grass rows in agroforestry might have not been exactly the same since the open grassland plots were three years younger than the grass rows in the agroforestry system (see discussion).

2.4 Soil characteristics

General soil characteristics were measured from samples taken at each sampling location with a soil auger within the depth of 0−0.3 m in summer 2016. The soil samples were dried at 40 °C for five days and passed through a 2-mm sieve. Soil texture was determined using the pipette method with pre-treatments for removing organic matter, iron oxide and carbonate for soils with pH ≥ 6 (Kroetsch and Wang 2008). Soil bulk density was measured using the soil core method (Blake and Hartge 1986) for 0−0.3-m depth in one plot each of the agroforestry and open grassland systems to minimize disturbance by the dug soil pits. For the top 0.05-m depth, bulk density was determined in all sampling locations. Soil pH was measured with a soil:water ratio of 1:4. Soil organic C and total N were determined using a CN analyzer (Elementar Vario El; Elementar Analysis Systems GmbH, Hanau, Germany); for soil samples with pH ≥ 6.0 pre-treatment for the removal of carbonates was performed (Harris et al. 2001). The effective cation exchange capacity (ECEC) was determined by percolating the soil with unbuffered 1 mol L^{-1} NH_4Cl followed by analysis of cations in the percolate using an inductively coupled plasma-atomic emission spectrometer (ICP-AES; iCAP 6300 Duo VIEW ICP Spectrometer, Thermo Fischer Scientific GmbH, Dreieich, Germany). Soil base saturation was calculated as the percentage of exchangeable bases of the ECEC.

2.5 Plant-available N

Plant-available N was measured six times (March, April, May, June, August, October 2016) for Histosol and Anthrosol soils and four times (March, May, August, November 2016) for Cambisol soil, using the buried bag method (Hart et al. 1994). On each measurement period, two intact soil cores were collected in the top 0.05 m at each sampling location; one was immediately extracted for mineral N in the field (T_0) and one was put in a usual plastic bag and incubated *in situ* in the hole that occurred from taking the soil core for six to eight days before extraction (T_1). The soil from each core was extruded into a plastic bag, mixed well, and stones and large organic materials were removed. A subsample was put into a prepared bottle containing 150 mL 0.5 mol L^{-1} K_2SO_4. Bottles with soil and extractant were shaken for one hour upon arrival in the laboratory, and the extracts were filtered through pre-washed filter papers; the filtrates were frozen immediately until analysis. Gravimetric moisture content, determined from the remaining soil of each soil core by oven-drying at 105 °C for at least 24 hours, was used to calculate the dry mass of the fresh soil extracted in the field. Concentrations of extractable mineral N were measured using continuous-flow injection colorimetry (AA3; SEAL Analytical GmbH, Norderstedt, Germany) by a salicylate and dicloroisocyanuric acid reaction for NH_4^+ (autoanalyzer method G-102-93) and by a cadmium reduction method with NH_4Cl buffer for NO_3^- (autoanalyzer method G-254-02). Plant-available N was calculated as the difference between T_1 and T_0 mineral N (NH_4^+ + NO_3^-), expressed as the net soil N mineralization rate. For calculation of the total net N mineralization during the growing season, the trapezoidal rule was applied between net N mineralization rates and time intervals of measurement periods. Total N supply available for plant uptake, as used in previous studies on NRE in grassland and forest ecosystems (Keuter et al. 2013, Schmidt et al. 2015), was total net N mineralization rates during the growing period (i.e. March−November) plus the annual N deposition values (12 kg N ha^{-1} year^{-1} for the site with Histosol and Anthrosol soils, and 15 kg N ha^{-1} year^{-1} for the site with Cambisol soil; Kruit et al. 2014).

2.6 Plant-available P

The sum of resin- and bicarbonate-extractable P was used as the index of plant-available P (Cross and Schlesinger 1995). This was measured on the T_0 soil samples used for net N mineralization. Resin-extractable P was determined by putting 0.5 g of air-dried, sieved soil into a centrifuge tube containing 30 mL deionized water and 1 g of anion exchange resin (DOWEX 41801 analytical grade; Serva Electrophoresis GmbH, Heidelberg, Germany) contained in a teabag. The centrifuge tube was shaken overnight. After washing the soil off the teabag with distilled water, the teabag was shaken overnight with 20 mL 0.5 mol L^{-1} HCl to extract the resin-adsorbed P. The remaining soil in the centrifuge was further extracted by shaking overnight with 30 mL 0.5 mol L^{-1} $NaHCO_3$. The extracts were frozen immediately until analysis. P concentrations of both extraction steps were then measured using ICP-AES (as above). The NRE calculation for each sampling location used the average of all P measurements during the growing season.

2.7 Nutrient response efficiency (NRE)

The parameters used for NRE are the harvested aboveground biomass of grasses or trees, plant-available N and P, and the soil exchangeable bases (Ca, Mg and K), described above. As I expect exchangeable cations (Ca, K, Mg) and plant-available P to not differ much between consecutive years on the extensively used grasslands in this study, the measured values from 2016 were used to also calculate NRE for 2017 for Histosol and Anthrosol soils. Since the Cambisol soil was fertilized in April 2017 (Table 1), measured values from the year before could not be used and thus NREs for 2017 for this soil type were not calculated.

Harvestable aboveground biomass of grasses and trees (wood and leaf litter production) was provided by other research groups of the SIGNAL project (Swieter et al. unpublished data, Malec et al. unpublished data; Table A2). These groups used the same method for harvesting. Grass biomass of a specified area was harvested at each sampling location with electric garden scissors. For the trees allometric equations for wood production (measured in January 2017, Verwijst and Telenius 1999) and litter traps (with an area of 0.10 m^2, placed at each sampling location in the agroforestry system) for leaf litter production were used.

Biomass production is given on the basis of an area, thus soil nutrients are treated likewise. It is important to note that for plant-available nutrients in the soil, the depth for which these indices are measured is arbitrary as long as this depth is uniform for the management systems being compared (e.g. Hiremath and Ewel 2001, Schmidt et al. 2015). Therefore, when comparing values of NRE from different studies, one should adjust the values according to the soil depth of measurements. Since the aim is to compare management systems, it is important that the conversion of nutrient element content per soil mass basis to an area basis must use an equal amount of soil mass, e.g. by varying the soil depth or, similarly, by using the soil bulk density of the reference system, in order to avoid the confounding effects of possible differences in soil masses within a certain depth (e.g. Allen et al. 2015). In this regard, it was first tested statistically if there were differences in soil bulk densities between the agroforestry and open grassland systems for each soil type (see Statistical analysis). As this was not the case, the average soil bulk density in the top 0.05 m for each soil type was used to convert soil nutrient contents from mass basis to area basis.

2.8 Soil water sampling and nutrient concentration in soil water

One month before the start of soil water sampling one suction cup lysimeter was installed (P80 ceramic, maximum pore size 1 µm; CeramTec AG, Marktredwitz, Germany) at each sampling location at 0.6-m depth. The lysimeters were connected to glass bottles stored in insulated boxes that were placed within the tree rows. Before samples were collected for the first time hoses were flushed through once with soil water. The soil water was sampled monthly from April 2016 to April 2017 by applying a suction of 600 hPa for one week. During some periods, no water could be collected due to frost (December 2016–January 2017 for Histosol and Anthrosol soils, January–February 2017 for Cambisol soil) and because of dry soil conditions (July and September 2016 for Histosol and Anthrosol soils, August–November 2016 for Cambisol soil). The water samples were transported to the laboratory immediately following collection and stored frozen at -18 °C until analysis.

Total dissolved N (TDN), NH_4^+, NO_3^-, and PO_4^{3-} were measured via continuous flow injection colorimetry (as above). TDN was determined by ultraviolet-persulfate digestion followed by hydrazine sulfate reduction (autoanalyzer method G-157-96), for NH_4^+ and NO_3^- see above. Dissolved organic nitrogen (DON) was calculated as the difference between TDN and NH_4^+ + NO_3^-. For determination of PO_4^{3-} concentration (=$P_{inorganic}$) a reaction with molybdate, antimony and ascorbic acid to produce a phosphate-molybdenum blue complex was performed (autoanalyzer Method G-092-93). Exchangeable cations (Ca, Mg, K, Na) and total P were measured using an ICP-AES (as above). For $P_{organic}$, the difference between total P and $P_{inorganic}$ was calculated (Kruse et al. 2015). Instruments' detection limits were: 13 µg NH_4^+-N L^{-1}, 5 µg NO_3^--N L^{-1}, 20 µg TDN-N L^{-1}, 0.004 µg PO_4^{3-} L^{-1}, 3 µg Ca L^{-1}, 3 µg Mg L^{-1}, 50 µg K L^{-1}, 30 µg Na L^{-1}, and 10 µg P L^{-1}.

2.9 Ratios of nutrient/Na concentrations

Sodium is not an essential nutrient and is not actively assimilated by the vegetation (Subbarao et al. 2003). Changes in soil water Na^+ concentration are thus mainly caused by the water balance, assuming that within one soil type the Na^+ input (through weathering and deposition) does not vary significantly. Therefore, to evaluate the role of vegetation uptake on nutrient concentrations in the soil solution, nutrient/Na ratios were calculated. The effect of water was thus excluded. Similar ratios including Na have been used as indices before e.g. the $Ca^{2+/}Na^+$ ratio to estimate weathering rates (Bailey et al. 2003), the Na/nutrient ratio to estimate element leaching from the crown of trees (Ulrich 1991) and the Na/(Na + Ca) ratio to distinguish the source of cations in stream water (Markewitz et al. 2001). An increase in the nutrient/Na ratio indicates an enrichment of the respective nutrient, relative to Na; a decrease in the ratio indicates a removal of the nutrient, relative to Na.

2.10 Water balance and calculation of nutrient leaching fluxes

The drainage water flux was modelled using the soil water module of the model system Expert-N (version 5.1, Priesack 2006) which was parametrized with site specific conditions i.e. climate data, soil characteristics, and site management. Meteorological data (air temperature, precipitation, relative humidity, wind speed, solar radiation) were obtained from stations at the two study sites (Markwitz et al. unpublished data). Soil characteristics were taken from Göbel et al. (2018). Data for soil temperature (°C) and soil moisture (volumetric) were measured continuously using sensors (SM300, UP GmbH, Ibbenbüren, Germany) installed in one plot each (tree rows and grass rows at the respective distances) in 0.3- and 0.5-m depth at the Histosol and Cambisol soils. For Anthrosol soil no sensors were installed.

In Expert-N the water balance of the soil-plant-atmosphere system considers five different components:

$$\Delta W + D = P - R - ET$$

where ΔW is the mass change of soil water, D drainage water below the rooting zone, P precipitation, and R runoff due to the sites' slope. ET, actual evapotranspiration, is calculated as:

$$ET = I + E + T$$

where I is the interception of water by plant foliage, E the actual evaporation from the soil and T the actual transpiration from the plant. To obtain E and T, at first the potential evapotranspiration is calculated with a Penman-Monteith approach (Walter et al. 2005). Vertical water movement is simulated using the Richards equation (HYDRUS model, Šimunek et al. 1998).

The hydraulic functions were parameterized using measured soil textures and water retention curves from literature (Mualem 1976, van Genuchten 1980, Sponagel 2005). Biomass production for grassland was estimated using the Hurley-Pasture model (Thornley 1998) and for trees using the interpolation approach of the LEACHN model (Hutson and Wagenet 1992). Both models were calibrated with measured aboveground biomass production from Swieter et al. (unpublished data) for Histosol and Anthrosol soils and from Malec et al. (unpublished data) for Cambisol soil (Table A2). A novel agroforestry module was implemented, which considers shading by the tree rows by means of reducing the radiative input onto the grass rows. This reduction is calculated using the solar zenith and azimuth angle, height, width and extinction coefficient k of the tree rows, as well as the distances of the different sampling locations from the tree rows in the grass rows. The equation for this calculation is the Beer-Lambert law:

$$I = I_{in} * e^{(k * LAI_{eff})}$$

where I is the radiative input for the grassland model, and I_{in} the measured solar radiation without shading. LAI_{eff} is the effective leaf area index: the simulated leaf area index of the Expert-N tree model is modified considering the path of the light through the tree row.

All models were optimized by comparing data of grass production and biomass production in the tree row with modelled biomass data, this being a good indicator for evaluating the overall performance of a model (Klein et al. 2017). Furthermore, modelled soil water content was compared visually with data from the soil moisture sensors (Histosol and Cambisol soils) and with measured soil moisture content at 0.05-m depth (Anthrosol soil). Monthly nutrient leaching fluxes were calculated by multiplying nutrient concentrations in the monthly sampled soil water with the total drainage flux of the respective month.

2.11 Gross rates of soil-N cycling

To determine gross rates of soil-N cycling processes (i.e. gross N mineralization, gross nitrification, N immobilization and DNRA), the ^{15}N pool dilution technique on intact soil cores was performed in the top 0.05-m depth (Davidson et al. 1991). Five intact soil cores per sampling location were extruded next to each other.

Sampling at the site with Histosol and Anthrosol soils was performed in April 2017. Two soil cores per sampling location were transported to the lab within three hours after sampling and processed there (^{15}N-T_0). Upon arrival the soil of each core was extruded, mixed thoroughly and large roots, stones and woody debris removed. The ^{15}N-T_0 cores were then sprinkled each with 5 mL of either $(^{15}NH_4)_2SO_4$ with 13 µg^{15}N/mL or $K^{15}NO_3$ with 14 µg^{15}N/mL with 95% ^{15}N enrichment. After ten minutes a portion of soil was placed in a prepared plastic bottle containing 150 mL 0.5 M K_2SO_4. To assure a complete mixture of soil and solution, bottles were shaken for an hour before the solution was filtered through pre-washed filter papers. Two additional soil cores were injected directly in the field with either $(^{15}NH_4)_2SO_4$ or $K^{15}NO_3$ (^{15}N-T_1). These ^{15}N-T_1-cores were incubated *in situ* for one day in plastic bags to prevent rain from entering and to allow for air exchange and were then extracted the same way as ^{15}N-T_0 cores. The fifth core was used for determination of N background values by extracting the same way as the labelled cores. Gravimetric moisture content was measured from each core by oven-drying a portion of the remaining soil at 105°C. Extracts were kept frozen at -18°C until analysis. The site with Cambisol soil was sampled in July 2015. Samples from this site were processed the same way, with the only difference that both, ^{15}N-T_0 and ^{15}N-T_1 cores, were injected directly in the field, two with 5 mL $(^{15}NH_4)_2SO_4$ with 22 µg^{15}N/mL and two with $K^{15}NO_3$ with 26 µg^{15}N/mL.

Concentrations of extractable mineral N were measured using continuous flow injection colorimetry (as above). Soil-available N was calculated as the difference between T_1 and T_0 mineral N (NH_4^+ + NO_3^-), expressed as gross soil-N mineralization rate. ^{15}N diffusion was used to determine the ^{15}N enrichment of NH_4^+ and NO_3^- pools as described in detail by Corre and Lamersdorf (2004). The analysis of ^{15}N was performed with an isotope ratio mass spectrometer (IRMS; Delta Plus, Finnigan MAT, Bremen, Germany). Calculations followed Davidson et al. (1991).

In order to measure microbial immobilization of NH_4^+ and NO_3^- and microbial biomass C and N a portion of remaining soil from the ^{15}N-T_1 cores was used for the chloroform ($CHCl_3$) fumigation-extraction method (Brookes et al. 1985). Samples were exposed to $CHCl_3$ for 5 days and were extracted afterwards with 0.5 M K_2SO_4 as described above (= fumigated). Organic C concentration in microbial biomass was measured with a total organic carbon analyzer (TOC-Vwp; Shimadzu Europa GmbH, Duisburg, Germany). Total N concentration was determined by persulfate digestion (Cabrera and Beare 1993). Microbial biomass was then calculated as the difference in extractable C (MBC) or N (MBN) between fumigated and unfumigated (^{15}N-T_1 cores) samples divided by $k_C = 0.45$ and $k_N = 0.68$ (Brookes et al. 1985).

2.12 Statistical analysis

Each parameter was first tested for normality in distribution (Shapiro-Wilk test) and homogeneity of variance (Levene test). If these criteria were not met, data were log- transformed and further analyses conducted with the transformed data. To assess the comparability of the initial soil conditions between the agroforestry and open grassland systems in each soil type, the differences in clay, silt and sand contents were tested using one-way analysis of variance (ANOVA, for data with normal distribution and homogenous variance) or Kruskal-Wallis H test (if otherwise). Soil texture did not differ (p = 0.38-0.75; Table 2) between the two management systems at each soil type. However, for the site with Cambisol soil the variability in clay contents among replicate plots was considerable so that clay content was used as covariate in the further statistical analysis for this site.

Measurements within the tree row and at various distances within the grass row were weighted according to the area they covered to calculate values for the agroforestry system as a whole, including both tree and grass rows. Considering the sampling locations as the center of the area they represented, the tree rows, 1-m, 4-m and 7-m distances within the grass rows covered an area of 4.95, 3.25, 3 and 3 m^2, respectively, in the Histosol and Anthrosol soils. Thus, the area-weighting factors were 0.35 for the tree row, 0.23 for the 1 m, 0.21 for the 4 m, 0.21 for the 7 m. In the Cambisol soil, the area-weighting factors were 0.46 for the tree row, 0.31 for the 1 m and 0.23 for the 4 m (based on their represented areas of 4, 2.75 and 2 m^2). The area-weighted values were then summed to get one value for each replicate plot in the agroforestry system. For comparison between agroforestry and open grassland systems as a whole either Independent T test (normal distribution and homogenous variance) or Mann-Whitney-Wilcoxon test (if otherwise) for the Histosol and Anthrosol soils were used. For the Cambisol soil, an analysis of covariance (ANCOVA) with clay content as covariate was used. To test differences between components of agroforestry and open grassland per soil type a one-way analysis of variance (ANOVA) for normally distributed data with Fisher's least significant difference (LSD) test was used. For data, that were even after transformation non-normally distributed, a Kruskal-Wallis-H test was performed. Since clay content differed considerably at the Cambisol site (Table 2), it was included as covariate in an analysis of covariance (ANCOVA) followed by Fisher's LSD test for this soil type. Where criteria for ANCOVA (normal-distribution and homogeneity of variances) were not met, a generalized linear model (GLM) with either Gaussian or Gamma distribution was used (based on quantile residuals). For the parameters with multiple sampling periods (plant-available N, P and water filled pore space), linear mixed-effect models (LME) with management system, including sampling locations of the agroforestry system, as fixed effect and time and replicate plots as random effects were used. The LME model included either 1) a variance function that allows different variances of the response variable for the fixed effects, 2) a first-order temporal autoregressive process that assumes the correlation between measurements decreases with increasing time difference, or 3) both if this improved the relative goodness of model fit based on the Akaike Information Criterion (Crawley 2007). Generalized linear mixed models were performed if even after transformation the assumption of normal distribution was not met for LME models. Hereafter, the model with a Shapiro-Wilk test closest to p = 0.05 was used. Spearman's rank correlation test was used to test relationships between gross rates of soil-N cycling and microbial parameters or soil biochemical characteristics across soil types. For all tests, the significance level was set at $p \leq 0.05$, except for a few parameters for which marginal significance ($p > 0.05 \leq 0.08$) was mentioned. All statistical analyses were performed with R version 3.3.0 (R Core Development team 2016).

Fitting of growth and NRE curves was first tried with the nonlinear least square method but no fit was achieved. Subsequently, the curves for P and K were put in manually and their parameters were repeatedly adjusted to achieve a maximal goodness of fit, evaluated by a Pearson correlation test between fitted and observed values. For N, no relationship was observed, which is the case when a nutrient has reached saturation levels and no longer responds to nutrient addition (Pastor and Bridgham 1999). NRE then is the constant value of mean productivity divided by nutrient availability.

3 Results

3.1 Soil characteristics and nutrient availability

Physico-chemical soil characteristics did not differ between the two management systems grassland agroforestry and open grassland (Table 2). Additionally, nutrient availability was generally comparable between management systems (Table 3). The only difference between management systems was the lower P and Mg availability in open grassland compared to grassland agroforestry for Histosol soil ($p \leq 0.08$, Table 3). Where nutrient availability differed among sampling locations in grassland agroforestry for Histosol and Anthrosol soils, it was generally lower in the tree row than in the grass row (Table A1). Nutrient availability in open grasslands – in both soils – was comparable to that in the agroforestry grass row. An opposite pattern was found among sampling locations in the Cambisol soil − the tree row and open grassland had higher plant-available P and K than the agroforestry grass row ($p \leq 0.05$, Table A1).

Table 2 Soil characteristics of agroforestry and adjacent open grasslands on three soil types in central Germany, measured in the top 0.3 m in 2016.

Soil type	Management system	Sampling location	Texture[1] (% Sand/ Silt/ Clay)	Soil pH (1:4 soil H_2O ratio)	Soil organic C (kg C m^{-2})	Total N (kg N m^{-2})	Soil C:N ratio	Effective cation exchange capacity ($mmol_c$ kg^{-1})	Base saturation (%)
Histosol soil	Agroforestry	tree row	38/46/16	4.8 ± 0.2	4.10 ± 0.78	0.15 ± 0.02	27.1 ± 2.3	185 ± 16	79 ± 8
		1 m	42/39/19	5.0 ± 0.2	4.64 ± 0.20	0.19 ± 0.00	24.7 ± 0.9	236 ± 14	89 ± 5
		4 m	46/36/18	4.9 ± 0.1	6.27 ± 1.19	0.24 ± 0.03	25.6 ± 2.0	220 ± 14	91 ± 2
		7 m	47/33/20	4.9 ± 0.1	6.81 ± 1.44	0.27 ± 0.05	25.4 ± 1.0	270 ± 35	93 ± 1
	Open		54/32/14	5.1 ± 0.1	5.14 ± 1.50	0.22 ± 0.06	22.9 ± 0.7	237 ± 38	95 ± 0
Anthrosol soil	Agroforestry	tree row	53/39/8	5.8 ± 0.0	1.53 ± 0.09	0.09 ± 0.00	17.4 ± 0.1	65 ± 5	95 ± 1
		1 m	51/40/9	5.9 ± 0.1	2.02 ± 0.16	0.12 ± 0.00	16.9 ± 1.1	88 ± 9	97 ± 1
		4 m	56/37/7	5.9 ± 0.0	1.94 ± 0.64	0.12 ± 0.04	16.1 ± 0.6	91 ± 19	97 ± 0
		7 m	52/38/10	5.9 ± 0.0	1.98 ± 0.23	0.11 ± 0.01	17.5 ± 2.0	90 ± 8	98 ± 0
	Open		57/32/11	6.0 ± 0.0	1.44 ± 0.03	0.09 ± 0.01	16.2 ± 1.7	80 ± 19	98 ± 1
Cambisol soil	Agroforestry	tree row	29/47/24	5.6 ± 1.2†	0.84 ± 0.18	0.09 ± 0.01†	9.3 ± 0.8	83 ± 9†	96 ± 1
		1 m	35/49/16	6.5 ± 0.3	0.97 ± 0.05	0.10 ± 0.01	10.0 ± 0.3	73 ± 7	95 ± 1
		4 m	35/50/15	6.6 ± 0.3	1.00 ± 0.05	0.10 ± 0.01	9.9 ± 0.2	112 ± 37	96 ± 2
	Open		50/28/23	5.3 ± 0.4	0.79 ± 0.06	0.09 ± 0.01	9.3 ± 0.3	77 ± 13	98 ± 1

Means ± standard errors (n = 3 for Histosol and Anthrosol soils, n = 4 for Cambisol soil) within each soil type showed no significant differences among sampling locations of agroforestry (i.e. tree rows and distances from the tree rows to the grassland rows) and open grassland (one-way ANOVA with Fisher's LSD test at $P \leq 0.05$ or Kruskal-Wallis test with multiple comparison extension at $p \leq 0.05$ for Histosol and Anthrosol soils; ANCOVA or GLM with Fisher's LSD test at $p \leq 0.05$ for Cambisol soil).

[1] Differences between sampling locations cannot be tested using ANCOVA because of multicollinearity, i.e. the effect of the different distances to the tree row cannot be statistically distinguished from the effect of clay content.

Table 3 Plant-available nutrients and nutrient response efficiencies (NRE) in agroforestry (AF) and adjacent open grasslands on three soil types in central Germany, measured in the top 0.05 m.

Soil type	Year	Management system	N (g N m^{-2} year)	P	K	Ca	Mg
				(g nutrient m^{-2})			
Histosol soil	2016	AF	11.1 ± 2.2 A	4.6 ± 0.4 A*	3.2 ± 0.3 A	92 ± 3 A	4.2 ± 0.2 A
		Open	12.7 ± 3.3 A	3.6 ± 0.2 B*	3.1 ± 0.5 A	109 ± 18 A	3.5 ± 0.1 B
Anthrosol soil	2016	AF	4.8 ± 0.8 A	4.3 ± 0.3 A	2.4 ± 0.2 A	56 ± 4 A	2.7 ± 0.0 A
		Open	4.8 ± 0.8 A	4.2 ± 0.1 A	2.2 ± 0.3 A	56 ± 14 A	2.5 ± 0.6 A
Cambisol soil	2016	AF	2.8 ± 0.7 A	6.3 ± 0.5 A	12.3 ± 0.5 A	99 ± 13 A	6.0 ± 1.0 A[1]
		Open	5.1 ± 1.8 A	7.9 ± 0.6 A	14.0 ± 1.9 A	90 ± 17 A	4.5 ± 0.6 A
	Year		NRE N (kg biomass m^{-2} $year^{-1}$/ kg N m^{-2} $year^{-1}$)	NRE P	NRE K	NRE Ca	NRE Mg
Histosol soil	2016	AF	82 ± 5 A	146 ± 15 A	291 ± 47 A	8 ± 2 A	198 ± 38 A
		Open	55 ± 21 A	160 ± 19 A	195 ± 45 A	5 ± 1 A	161 ± 17 A
	2017	AF	-	200 ± 16 A	445 ± 68 A*	12 ± 3 A	298 ± 55 A*
		Open	-	91 ± 15 B	108 ± 23 B*	3 ± 0 B	91 ± 11 B*
Anthrosol soil	2016	AF	140 ± 16 A	129 ± 8 A	255 ± 24 A	10 ± 1 A	217 ± 8 A
		Open	108 ± 30 A	114 ± 12 A	225 ± 39 A	10 ± 3 A	214 ± 65 A
	2017	AF	-	197 ± 13 A	410 ± 40 A	16 ± 0 A	348 ± 27 A
		Open	-	98 ± 11 B	196 ± 39 B	8 ± 1 B	169 ± 23 B
Cambisol soil	2016	AF	1573 ± 927 A	142 ± 22 A	77 ± 8 B*	10 ± 2 A	160 ± 34 B
		Open	419 ± 156 A	168 ± 6 A	100 ± 15 A*	16 ± 2 A	309 ± 39 A

Means ± standard errors (n = 3 for Histosol and Anthrosol soils, n = 4 for Cambisol soil) followed by a different letter indicate significant differences between the two management systems per year within each soil type (Independent T test or Mann-Whitney-Wilcoxon test at $p \leq 0.05$ for Histosol and Anthrosol soils; ANCOVA with Fisher's LSD test at $p \leq 0.05$ for Cambisol soil)

[1] Differences between grassland agroforestry and open grassland cannot be tested using ANCOVA because of multicollinearity, i.e. the effect of the different management systems cannot be statistically distinguished from the effect of clay content

* $p \leq 0.08$

3.2 Biomass production and nutrient response efficiency

In 2016, biomass production between the two management systems only differed in the Cambisol soil with more biomass in the open grassland than in the agroforestry system ($p = 0.04$; Table A2). Grass biomass production among sampling locations was higher in the open grassland than at 1-m distance in the grass row of the agroforestry for the Cambisol soil ($p = 0.04$, Table A2). Tree biomass production (from the first year of the second rotation) for Histosol and Anthrosol soils was higher than grass production at 1-m distance for the Histosol soil and the highest compared to grass production for the Anthrosol soil ($p \leq 0.05$, Table A2).
In 2017, biomass production of grassland agroforestry was higher than that of open grassland for all sites due to significantly higher biomass production of the trees than all sampling locations in the grass row and open grassland ($p \leq 0.05$, Table A2). Grass biomass production among sampling locations in agroforestry and open grassland was comparable per site.

Nutrient response efficiencies (NRE) for 2016, similarly to biomass production, only differed between the two management systems in the Cambisol soil. Here, Ca response efficiency was marginally higher ($p = 0.07$), and Mg response efficiency was higher ($p = 0.01$) in the open grassland than in the agroforestry system (Table 3). However, when comparing agroforestry sampling locations and the open grassland system, NRE patterns differed in each soil type (Table A2): For Histosol and Anthrosol soils, NREs were mostly higher in the tree row than in the grass row of agroforestry ($p \leq 0.05$) whereas the open grassland was often comparable to the pertaining sampling locations of agroforestry. For the Cambisol soil, the Mg response efficiency was lower at the 1-m distance in the agroforestry grass row than in the open grassland ($p = 0.04$).
In 2017, NREs for Histosol and Anthrosol soils were generally higher for grassland agroforestry and lower for open grassland than values from 2016 (Table 3). NREs of both soil types were higher for agroforestry than open grasslands (Table 3, $p < 0.08$). Among sampling locations a clear pattern was seen for this year, with the trees always having significantly higher NREs than the grass rows or open grassland (Table A2, $p < 0.05$). Based on the relationship of productivity with plant-available nutrients, both management systems in all soil types were N saturated in 2016, but responded to increased availability of P and K (Figs 5 a, c, e). The NREs were either at the optimum range (i.e. P for all soils and K for Histosol and Anthrosol soils; Fig 5 d and f) or beyond the optimum range (i.e. N for all sites and K for Cambisol soil; Figs 5 b and f). The NREs of P and K in 2017 tended to have a higher optimum than the NREs of these nutrients in 2016 (Figs 5 d and f).

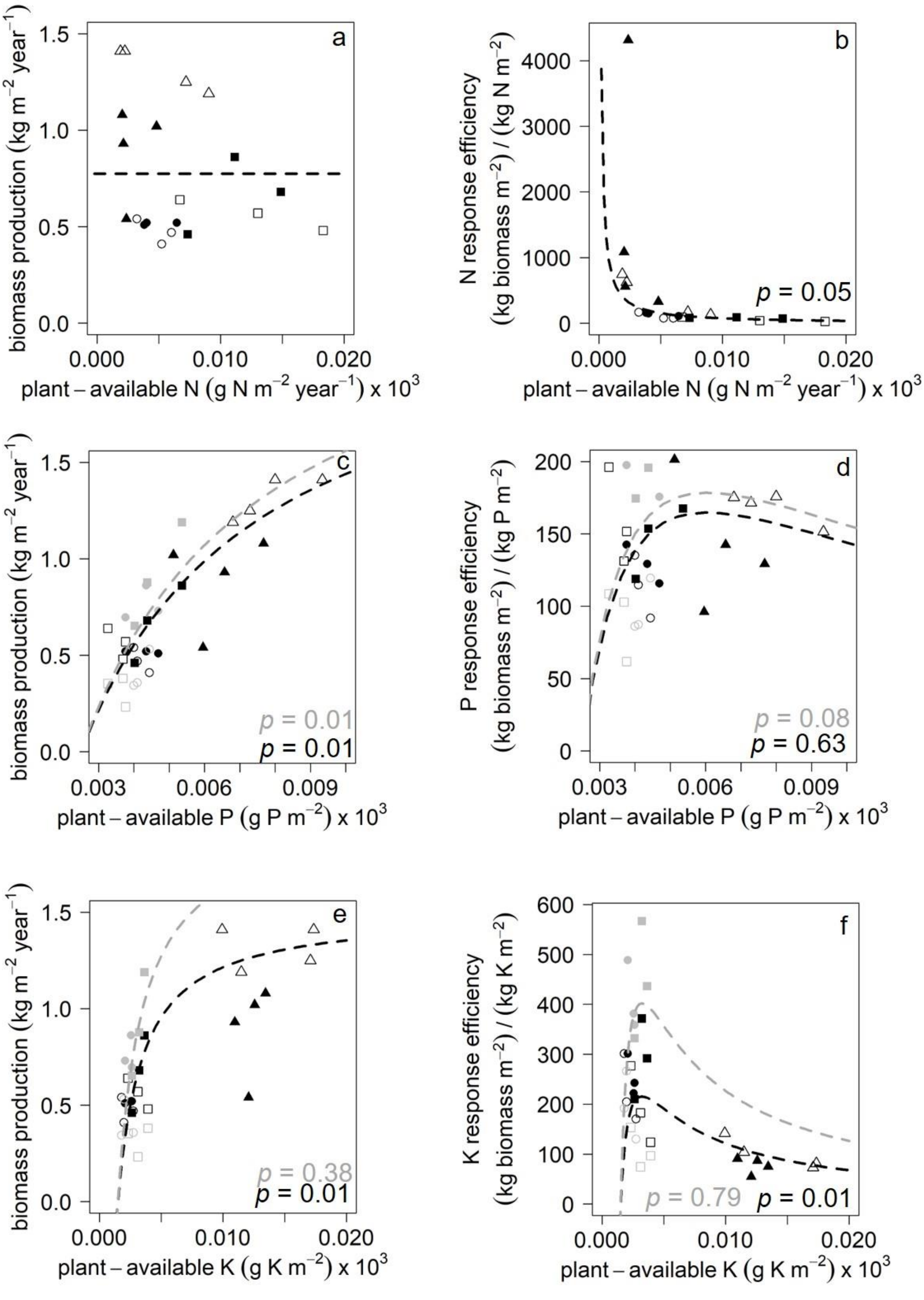


Fig 5 Aboveground biomass production and nutrient response efficiency with plant available nitrogen (a, b), phosphorus (c, d) and potassium (e, f) in grassland agroforestry (filled symbols) and adjacent open grasslands (empty symbols) in Histosol (■), Anthrosol (•) and Cambisol soils (▲) in black for 2016 and in grey for 2017. The curves for P and K were put in manually and their parameters repeatedly adjusted to achieve a maximal goodness of fit, evaluated by a Pearson correlation test between fitted and observed values (n = 20 for 2016, n = 12 for 2017). For N, no relationship was observed, which is the case when a nutrient has reached saturation levels and no longer responds to nutrient addition (Pastor and Bridgham 1999).

3.3 Water balance and water drainage fluxes

At the beginning of the study year (April 2016) transpiration was greater in the grass rows than in the tree rows (Fig 6). This changed with full foliation of the trees until defoliation in autumn 2016 stopped transpiration. Water drainage rates were generally low during the summer and increased strongly during winter, when transpiration was minimized, of both trees and grasses (Fig 6).

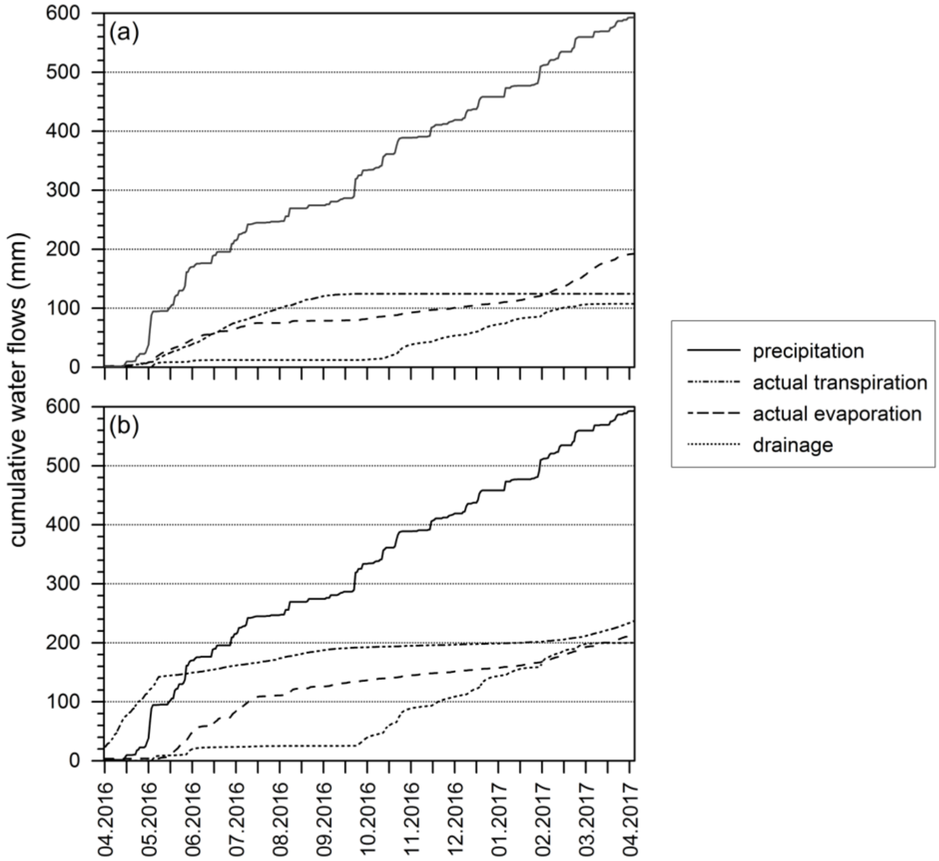


Fig 6 Cumulative precipitation, transpiration, evaporation, and water drainage during the study year for (a) tree row and (b) grass row at 4-m distance from the tree row at the Histosol soil site.

For all soil types tree rows displayed high interception rates resulting in water drainage fluxes that were considerably lower compared to the grass rows (Table 4). Evapotranspiration of grass rows at 1-m distance was smaller compared to the grass rows at 4-m distance to the tree rows at the Cambisol soil because of shading effects of the trees. This resulted in the highest water drainage of the alley cropping system at this distance (Table 4). This effect was not visible at the Histosol and Anthrosol soils because of the lower height of trees due to the harvest in the winter before the measurements, and consequently only limited shading of the grass rows.

Table 4 Simulated annual water balance components of tree rows and at several distances to the tree rows within grass rows in temperate grassland agroforestry on three soil types in central Germany during the study period April 2016–April 2017.

Water balance components (mm yr^{-1})	Histosol soil precipitation (592 mm yr^{-1})				Anthrosol soil precipitation (592 mm yr^{-1})				Cambisol soil precipitation (520 mm yr^{-1})		
	tree row	1 m	4 m	7 m	tree row	1 m	4 m	7 m	tree row	1 m	4 m
Evapotranspiration	480	457	405	432	466	420	421	425	514	350	407
Transpiration	124	145	164	147	158	150	152	158	244	158	213
Evaporation	191	312	241	284	144	270	269	267	128	193	193
Interception	165	0	0	0	165	0	0	0	141	0	0
Water drainage	108	182	205	196	127	199	206	201	24	187	129
Δ Water storage	4	-47	-18	-36	-1	-27	-35	-34	-18	-17	-16

3.4 Nutrient concentrations, ratios of nutrient/Na concentrations in soil water and leaching losses

N-concentrations in soil water only differed for the Cambisol soil, where NH_4^+, NO_3^- and TDN concentrations were higher at 4-m distance than at the tree row or at 1-m distance ($p \leq 0.05$, Table 5). For Histosol and Anthrosol soils, concentrations of Ca^{2+}, Mg^{2+}, K^+, and Na^+ were generally highest in the tree row and decreased with greater distance to the tree row ($p \leq 0.05$, Table 5).

Nutrient/Na ratios for Histosol and Anthrosol soils were lower in the tree rows than in the grass rows and increased with increasing distance from the tree rows ($p \leq 0.05$, Table 6). For Cambisol soil, there were no differences in nutrient/Na ratios between tree and grass rows (Table 6).

At all three sites NO_3, Phosphate and base cation (Ca, K, Mg) leaching losses were highly variable throughout the study year (Fig 7). Tree and grass rows in general showed the same seasonal pattern of leaching losses with lower or negligible losses throughout the summer months and higher losses in winter and spring or after a strong downpour (e.g. in June for Histosol and Anthrosol soils, Fig 7). For most nutrients, leaching losses did not differ between tree rows and grass rows because of considerable temporal variability (Table A3). Sodium was the only element that had higher leaching losses in the tree rows of the Histosol and Anthrosol soils compared to losses at 4-m and 7-m distances in the grass rows; in Cambisol soil this pattern was opposite (Table A3).

Table 5 Nutrient concentrations in soil solution from a depth of 0.6 m under tree rows and several distances to the tree rows within grass rows in temperate grassland agroforestry on three soil types in central Germany from the study period April 2016–April 2017.

Nutrient (mg nutrient L^{-1})	tree row	1 m	4 m	7 m
	Histosol soil			
Ammonium	0.16 ± 0.01 a	0.30 ± 0.08 a	0.22 ± 0.02 a	0.22 ± 0.02 a
Nitrate	13 ± 7 a	9 ± 3 a	8 ± 5 a	14 ± 8 a
Dissolved organic N	4.4 ± 1.0 a	3.7 ± 1.3 a	3.8 ± 1.2 a	3.4 ± 0.7 a
Total dissolved N	17 ± 7 a	13 ± 4 a	13 ± 6 a	17 ± 9 a
Calcium	86± 15 ab	73 ± 28 a	46 ± 13 bc	37 ± 5 c
Magnesium	7.0 ± 1.2 ab	7.0 ± 2.2 a	4.4 ± 1.4 b	4.6 ± 0.3 b
Potassium	1.29 ± 0.24 a	1.31 ± 0.57 ab	0.54 ± 0.21 b	0.73 ± 0.21 ab
Sodium	31.2 ± 0.7 a*	12.0 ± 5.2 b*	4.8 ± 2.5 c	2.6 ± 0.9 c
Phosphate (µg PO_4^{3-} L^{-1})	3.9 ± 1.8 a	4.8 ± 2.7 a	2.5 ± 0.4 a	4.4 ± 1.5 a
$P_{organic}$ (µg P L^{-1})	11.1 ± 1.1 a	9.0 ± 4.5 a	9.9 ± 3.0 a	11.2 ± 5.8 a
	Anthrosol soil			
Ammonium	0.26 ± 0.23 a	0.23 ± 0.03 a	0.11 ± 0.03 a	0.10 ± 0.04 a
Nitrate	2 ± 1 a	4 ± 2 a	2 ± 1 a	2 ± 1 a
Dissolved organic N	1.1 ± 0.3 a	0.9 ± 0.3 a	1.1 ± 0.3 a	0.9 ± 0.2 a
Total dissolved N	4 ± 1 a	6 ± 2 a	4 ± 1 a	3 ± 1 a
Calcium	24 ± 7.67 a	19 ± 4.13 a	12 ± 2.51 b	9 ± 1 b
Magnesium	2.3 ± 0.8 a	2.0 ± 0.5 a	1.1 ± 0.1 b	1.0 ± 0.2 b
Potassium	0.48 ± 0.11 a	0.27 ± 0.02 b	0.21 ± 0.01 b	0.34 ± 0.16 ab
Sodium	11.5 ± 1.2 a	4.7 ± 1.1 b	3.0 ± 0.3 c	2.7 ± 1.1 c
Phosphate (µg PO_4^{3-} L^{-1})	3.9 ± 1.4 a	4.5 ± 0.1 a	3.9 ± 1.1 a	3.3 ± 1.7 a
$P_{organic}$ (µg P L^{-1})	5.2 ± 3.9 a	2.1 ± 2.1 a	1.7 ± 1.0 a	2.0 ± 1.0 a
	Cambisol soil			
Ammonium	0.06 ± 0.02 b	0.03 ± 0.01 c	0.11 ± 0.08 a	
Nitrate	0.15 ± 0.09 b	0.05 ± 0.01 b	2.00 ± 1.30 a	
Dissolved organic N	0.2 ± 0.1 a	0.1 ± 0.1 a	0.2 ± 0.1 a	
Total dissolved N	0.4 ± 0.2 b	0.2 ± 0.1 b	2.4 ± 1.5 a	
Calcium	19 ± 5 a	16 ± 4 a	11 ± 2 a	
Magnesium	9.0 ± 4.7 a	7.4 ± 4.4 a	3.6 ± 1.8 a	
Potassium	2.57 ± 0.86 a	1.73 ± 0.30 a	2.49 ± 0.23 a	
Sodium	3.3 ± 0.7 a	3.1 ± 1.3 a	2.3 ± 0.4 a	
Phosphate (µg PO_4^{3-} L^{-1})	4.4 ± 3.3 a	15.9 ± 5.9 a	6.3 ± 3.0 a	
$P_{organic}$ (µg P L^{-1})	50.9 ± 37.8 a	0.1 ± 0.1 a	1.8 ± 1.6 a	

Means ± standard errors (n = 3 for Histosol and Anthrosol soils, n = 4 for Cambisol soil) followed by a different letter indicate significant differences between sampling locations within each soil type (LME model or generalized mixed models with Fisher's LSD test at $p \leq 0.05$)
* $p = 0.07$

Table 6 Nutrient/Na concentration ratios in soil solution from a depth of 0.6 m under tree rows and several distances to the tree rows within grass rows in temperate grassland agroforestry on three soil types in central Germany from the study period April 2016−April 2017.

Nutrient/Na	tree row	1 m	4 m	7 m
	Histosol soil			
NH_4^+/Na^+	0.02 ± 0.01 bc	0.05 ± 0.03 c	0.10 ± 0.03 ab	0.13 ± 0.15 a
NO_3^-/Na^+	0.4 ± 0.2 b	0.9 ± 0.3 b	2.5 ± 1.7 ab	5.4 ± 1.4 a
Ca^{2+}/Na^+	2.8 ± 0.4 c	6.6 ± 1.0 b	15.2 ± 3.2 a	21.5 ± 9.2 a
K^+/Na^+	0.1 ± 0.0 b	0.2 ± 0.2 ab	0.2 ± 0.1 ab	0.3 ± 0.0 a
Mg^{2+}/Na^+	0.2 ± 0.0 c	0.7 ± 0.1 c	1.6 ± 0.3 b	2.6 ± 1.1 a
	Anthrosol soil			
NH_4^+/Na^+	0.02 ± 0.02 a	0.06 ± 0.01 a	0.04 ± 0.01 a	0.06 ± 0.02 a
NO_3^-/Na^+	0.2 ± 0.1 b	0.9 ± 0.3 a	0.7 ± 0.3 ab	0.7 ± 0.1 a
Ca^{2+}/Na^+	2.0 ± 0.5 b	4.2 ± 0.6 a	4.1 ± 1.3 a	5.0 ± 2.0 a
K^+/Na^+	0.0 ± 0.0 c	0.1 ± 0.0 bc	0.1 ± 0.0 b	0.1 ± 0.0 a
Mg^{2+}/Na^+	0.2 ± 0.1 b	0.4 ± 0.1 a	0.4 ± 0.1 a	0.4 ± 0.1 a
	Cambisol soil			
NH_4^+/Na^+	0.02 ± 0.01 a	0.01 ± 0.00 a	0.05 ± 0.03 a	
NO_3^-/Na^+	0.1 ± 0.0 a	0.0 ± 0.0 a	1.1 ± 0.5 a	
Ca^{2+}/Na^+	5.9 ± 0.4 a	7.2 ± 1.7 a	5.3 ± 0.5 a	
K^+/Na^+	1.2 ± 0.5 a	1.3 ± 0.7 a	1.3 ± 0.3 a	
Mg^{2+}/Na^+	2.2 ± 0.9 a	1.8 ± 0.5 a	1.4 ± 0.6 b	

Means ± standard errors (n = 3 for Histosol and Anthrosol soils, n = 4 for Cambisol soil) followed by a different letter indicate significant differences between sampling locations within each soil type (LME model or generalized mixed models with Fisher's LSD test at $p \leq 0.05$)

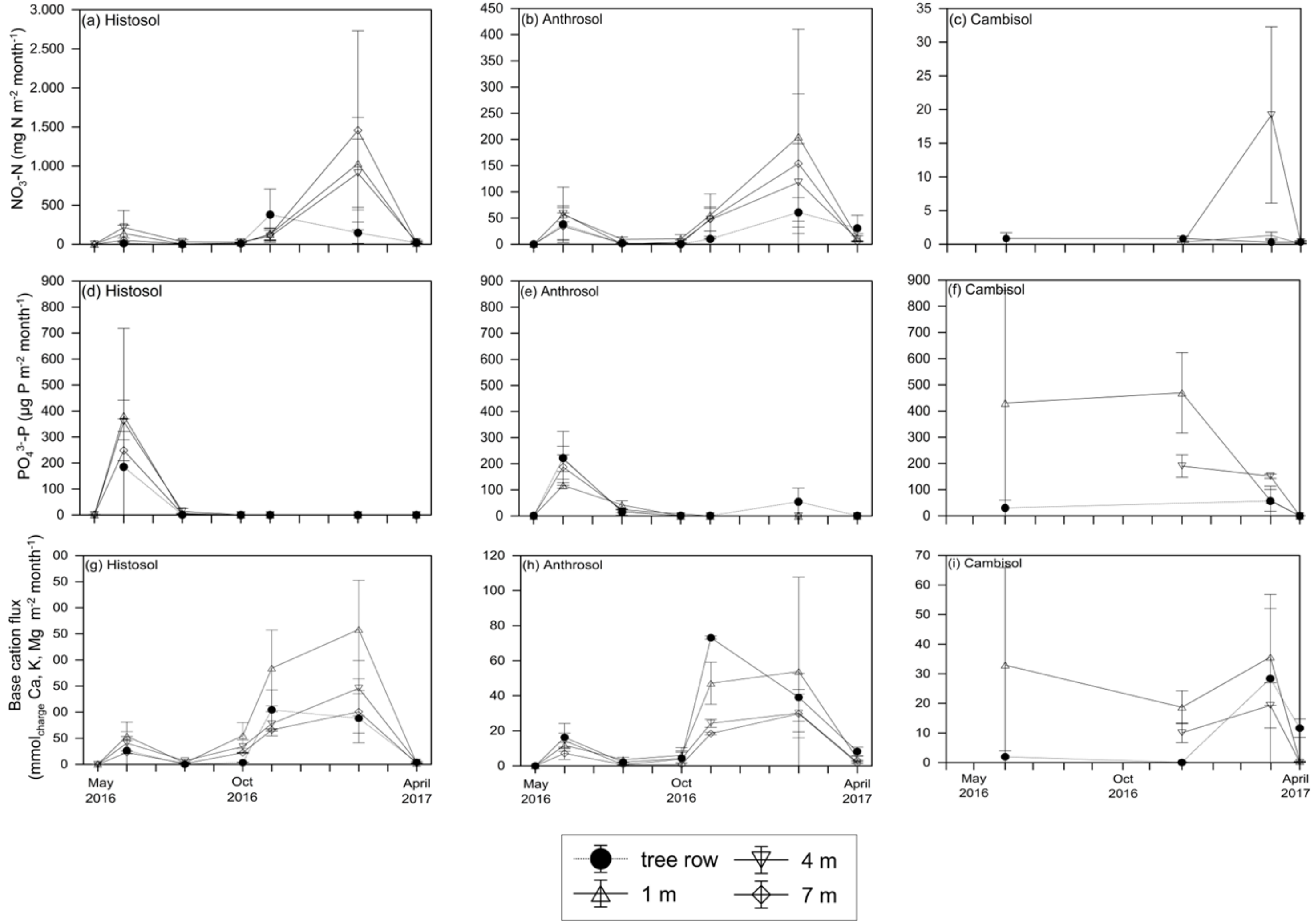


Fig 7 Nitrate (NO_3^--N; a–c), Phosphate (PO_4^{3-}-P; d–f), and base cation (Ca, K, Mg; g–i) leaching losses at 0.6-m depth under tree rows and several distances to the tree rows within grass rows in temperate grassland agroforestry on three soil types in central Germany (means ± standard errors, Histosol and Anthrosol soils n=3, Cambisol soil n=4).

3.5 Soil N cycling rates

Rates of NH_4^+ transformation processes were generally higher than NO_3^- transformation processes. There were no significant differences in gross rates of mineral N production (mineralization and nitrification) between sampling locations in grassland agroforestry and open grasslands within soil types (Table 7, $p > 0.05$). Microbial immobilization of NH_4^+ only differed for the Cambisol soil, where NH_4^+ immobilization was higher in open grassland than in the agroforestry grass row and lower in 4-m distance than in the tree row (Table 7, $p = 0.008$). For all soil types, DNRA did not differ per soil type ($p > 0.05$, Table 7) and had lower rates than NO_3^- consumption.

Distinguishable attributes in the soil-N cycling such as turnover times and microbial biomass were generally comparable between the two management systems and within agroforestry systems per soil type, respectively (Table 8). For Histosol and Anthrosol soils NH_4^+ turnover time was faster than NO_3^- turnover, whereas for Cambisol soil turnover times of NH_4^+ and NO_3^- were similar (Table 8). Microbial parameters such as MBN, MBC and microbial C:N did not differ for Histosol and Anthrosol soils (Table 8). Open grassland at the Cambisol soil had higher MBN than in the whole agroforestry system (Table 8, $p = 0.003$), but MBC and microbial C:N did not differ (Table 8).

Gross N mineralization rates were positively correlated with MBN which, in turn, was positively correlated with total N content, C:N ratio, effective cation exchange capacity (ECEC) and base saturation (data not depicted) for all soil types (Figs 8 a-d).

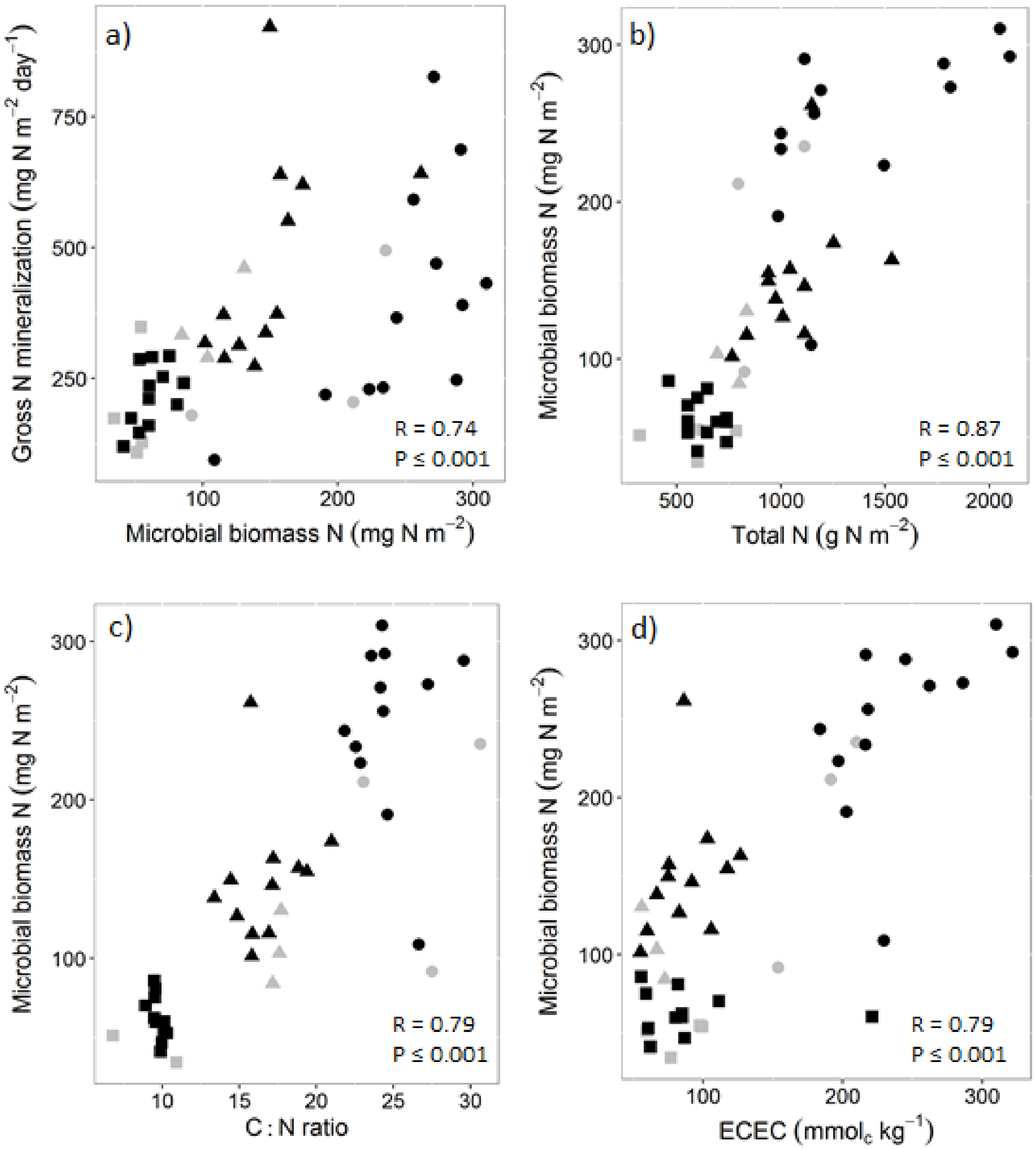


Fig 8 Relationships of gross N mineralization with (a) microbial biomass N, and microbial biomass N with (b) total N, (c) C:N ratio and (d) effective cation exchange capacity (ECEC) across grassland agroforestry and open grassland systems for three soil types (Histosol soil ●, Anthrosol soil ▲, Cambisol soil ■; black for grass, and gray for trees), assessed using Spearman's rank correlation test (n = 46).

Table 7 Gross rates of soil-N cycling in grassland agroforestry (AF) and adjacent open grasslands, measured in the top 0.05 m on three soil types in Lower Saxony, Germany.

Soil type	Management system	Sampling location	Gross N mineralization	NH_4^+ immobilization	Gross nitrification[1]	NO_3^- consumption[1]	Dissimilatory nitrate reduction to ammonium
					(mg N m^{-2} day^{-1})		
Histosol soil	AF	tree row	293 ± 101 a	266 ± 115 a	264	395	0.0 ± 0.0 a
		1 m	536 ± 225 a	415 ± 63 a	19	79	0.3 ± 0.3 a
		4 m	356 ± 118 a	676 ± 174 a	86 ± 33	89 ± 36	0.0 ± 0.0 a
		7 m	360 ± 74 a	323 ± 60 a	117 ± 44	190 ± 32	1.4 ± 1.4 a
	Open		343 ± 59 a	447 ± 134 a	91	178	0.0 ± 0.0 a
Anthrosol soil	AF	tree row	362 ± 51 a	346 ± 139 a	75 ± 58	168	31 ± 4 a
		1 m	414 ± 113 a	311 ± 73 a	230 ± 27	222 ± 28	39 ± 20 a
		4 m	522 ± 79 a	628 ± 311 a	141 ± 22	146 ± 31	95 ± 67 a
		7 m	627 ± 169 a	459 ± 111 a	213 ± 22	219 ± 26	32 ± 18 a
	Open		322 ± 29 a	294 ± 25	32	51	24 ± 19 a
Cambisol soil	AF	tree row	190 ± 55 a	335 ± 45 ab*	51 ± 14 a	23 ± 16 a	26 ± 10 a
		1 m	192 ± 38 a	206 ± 41 bc	59 ± 8 a	36 ± 11 a	13 ± 2 a
		4 m	214 ± 29 a	176 ± 36 c	90 ± 13 a	71 ± 10 a	11 ± 1 a
	Open		247 ± 19 a	412 ± 19 a	92 ± 24 a	96 ± 12 a	13 ± 3 a

Means ± standard errors (n = 3 for Histosol and Anthrosol soils, n = 4 for Cambisol soil) followed by a different letter indicate significant differences among sampling locations of agroforestry (i.e. tree rows and various distances within the grass rows) and open grassland (one-way ANOVA with Fisher's LSD test at $p \leq 0.05$ or Kruskal-Wallis H test with multiple comparison extension for Histosol and Anthrosol soils; ANCOVA or GLM with Fisher's LSD test at $p \leq 0.05$ for Cambisol soil)

[1] Cannot be tested statistically for Histosol and Anthrosol soils due to missing values of replicate plots

* $p = 0.08$

Table 8 Turnover times of soil mineral N and microbial N in grassland agroforestry (AF) and adjacent open grasslands, measured in the top 0.05 m on three soil types in Lower Saxony, Germany.

Soil type	Management system	Sampling location	NH_4^+ turnover (day)	NO_3^- turnover[1] (day)	Microbial N (mg N m^{-2})	MRT (day)	Microbial C (mg C m^{-2})	Microbial C:N
Histosol soil	AF	tree row	0.53 ± 0.02 a	0.68	4669 ± 1155 a	23 ± 6 a	50,051 ± 12,295 a	11.0 ± 1.1 a
		1 m	0.44 ± 0.28 a	13.40 ± 5.27	5814 ± 1500 a	16 ± 5 a	53,766 ± 14,285 a	9.3 ± 0.5 a
		4 m	0.47 ± 0.09 a	4.79 ± 3.66	6650 ± 485 a	11 ± 2 a	71,167 ± 5478 a	10.7 ± 0.4 a
		7 m	0.39 ± 0.03 a	2.17 ± 1.51	6553 ± 811 a	21 ± 3 a	74,963 ± 7622 a	11.5 ± 0.5 a
	Open		0.43 ± 0.09 a	1.97	6824 ± 624 a	17 ± 4 a	67,177 ± 6606 a	9.8 ± 0.3 a
Anthrosol soil	AF	tree row	0.73 ± 0.07 a	6.49 ± 5.00	4240 ± 535 a	15 ± 3 a	29,069 ± 2232 a	7.0 ± 0.4 a
		1 m	0.38 ± 0.02 ab	0.21 ± 0.03	5333 ± 494 a	18 ± 2 a	36,855 ± 7736 a	6.9 ± 1.0 a
		4 m	0.26 ± 0.02 b	0.43 ± 0.11	7198 ± 1722 a	15 ± 4 a	44,188 ± 10,528 a	6.2 ± 0.2 a
		7 m	0.35 ± 0.12 ab	0.29 ± 0.01	6264 ± 348 a	15 ± 3 a	45,008 ± 3200 a	7.3 ± 0.1 a
	Open		0.44 ± 0.11 ab	1.41	5260 ± 630 a	19 ± 4 a	38,111 ± 11,795 a	7.1 ± 1.5 a
Cambisol soil	AF	tree row	0.65 ± 0.26 a	0.77 ± 0.26 a	3382 ± 335 b	22 ± 6 a	41,661 ± 2515 a	13.0 ± 2.4 a
		1 m	0.54 ± 0.14 a	0.34 ± 0.14 a	3735 ± 330 b	21 ± 2 a	39,915 ± 1904 a	10.8 ± 0.5 a
		4 m	0.48 ± 0.09 a	0.28 ± 0.12 a	3885 ± 224 b	19 ± 3 a	42,310 ± 2294 a	10.9 ± 0.5 a
	Open		0.46 ± 0.08 a	0.49 ± 0.09 a	5390 ± 235 a	22 ± 2 a	48,603 ± 5966 a	8.9 ± 0.8 a

Means ± standard errors (n = 3 for Histosol and Anthrosol soils, n = 4 for Cambisol soil) followed by a different letter indicate significant differences among sampling locations of grassland agroforestry (i.e. tree rows and distances from the tree rows to the grass rows) and open grassland (one-way ANOVA with Fisher's LSD test at $p \leq 0.05$ for Histosol and Anthrosol soils; ANCOVA or GLM with Fisher's LSD test at $p \leq 0.05$ for Cambisol soil)

[1] Cannot be tested statistically for Histosol and Anthrosol soils due to missing values of replicate plots

4 Discussion

4.1 Can temperate grassland agroforestry be a sustainable alternative to open grassland in terms of soil nutrient availability and nutrient response efficiency?

The underlying hypothesis of this study was that grassland agroforestry systems have a higher NRE compared to open grasslands. Partially higher NREs in grassland agroforestry in comparison to open grassland at least under certain management conditions support this supposition. (Table 3). Where NREs in the present study were higher for grassland agroforestry than open grassland this resulted from significantly higher NREs of the tree rows than of the grass rows or open grassland due to the increased tree production in the second year after the harvest (Table A2). This increased biomass production also led to higher optimum NREs than in 2016 (Fig 6 d and f) suggesting that grassland agroforestry systems get more efficient with time (i.e. when the trees mature). It may be possible that the hypothesized higher NRE in grassland agroforestry compared to open grassland would also occur in systems with young trees when plant-available nutrient levels are lower than in this study. Additionally, positive interactions of agroforestry systems, e.g. trees acting as nutrient pump (Mosquera-Losada et al. 2011), are expected to also be more effective at those lower nutrient levels.

Regarding soil nutrients or water availability no indications for net effects of competition or complementarity were found between trees and grasses in these relatively young grassland agroforestry systems (at least in the top 0.05 m, Table A1). This possibly was due to both systems exhibiting optimum or saturated levels of soil nutrients (Figs 6 a−f). The grassland agroforestry systems in the current study were only 5- and 8-years old (i.e. formerly a fertilized cropland in the Cambisol soil, and open grassland management in the Histosol and Anthrosol soils, Table 1). Such high and comparable levels of soil nutrients between the two management systems could be a legacy of the previous management (Table A1). The regular application of digested residue from a biogas plant also contributed to the high nutrient status of both management systems at the Histosol and Anthrosol soils. This is also seen by the NRE values far beyond the optimum N availability in the present study (Fig 6 b). Studies in a Mongolian grassland (Yuan et al. 2006) and in a tropical tree plantation (Hiremath and Ewel 2001) even showed a monotonic decrease of NRE with increasing soil N availability instead of a unimodal efficiency. This suggests that a system is at nutrient saturation and beyond the optimum NRE (Pastor and Bridgham 1999). Although the availability of some nutrients (P, K or Mg) differed between the tree and grass rows within the agroforestry system, these nutrient levels were still comparable with those in the open grassland systems (Table A1), again suggesting legacy effects of the years of open grassland management prior to the establishment of the agroforestry systems. The young age of the investigated systems could be another reason why in the present study no changes in nutrient availability between trees and grass were detected. Previous studies have found no change in soil properties in such short time-spans, but only after at least ten years of systems' establishment (Oelbermann and Voroney 2007, Pardon et al. 2017). It is assumed that there are no other published studies yet on how soil nutrients and NRE change in grassland agroforestry systems at decadal time scales after more than just one rotation of the trees as in this study. Grassland composition most likely changes as the systems become older due to e.g. the shade influence of the trees. Therefore, it would be worth looking at changes in species composition and its possible influences on plant-available nutrients and NRE.

The benefits of agroforestry, based on NREs, were more apparent when the grass rows were wider (i.e. 48-m wide vs. 9-m wide). Lower biomass of grasses close to the tree row at the Cambisol soil site in a few cases led to lower NREs compared to the open grassland system. The two exceptions observed, i.e. higher NREs for Ca and Mg under open grassland as compared to grassland agroforestry in the Cambisol soil in 2016 (Table 3), were due to the low biomass production in the 1-m grass row in 2016 (Table A2). As Ca and Mg availability in the soil were comparable between the tree row and the grass rows (Table A1), the low biomass in the 1-m grass row could possibly be due to the effect of tree shadow on the grasses nearest to the tree row (Ehret et al. 2015). In this Cambisol soil, the NREs for Ca and Mg for the whole grassland agroforestry system were strongly influenced by the decrease in biomass close to the tree row, since the whole grass row was only 9-m wide. The detrimental effect of a narrow width was also visible with a significantly lower biomass production of the overall grassland agroforestry system compared to open grassland at this site in 2016 (Table A2). Such narrow width of alley cropping seems to be disadvantageous, especially when the trees are tall, as was the case at this site because the trees had already regrown for two years since the first cut. In the other two soil types, the trees had been cut only one year ago and the grass rows were 48-m wide; hence, the low grass biomass close to the tree row was outweighed by the grass biomass and NREs further away, which were comparable to the open grassland management (Table A2). It should be taken into account that the width of machinery is also often wider than the 9-m wide grass rows in the Cambisol soil in this study; thus, alley cropping agroforestry systems should also consider the ease and cost efficiency of mechanized farm operations.

The findings suggest that the fast-growing trees in the agroforestry system were able to convert plant-available nutrients more efficiently into biomass than mature tree stands in Germany (Schmidt et al. 2015). Values of NREs in the tree row were equal or higher than those of a temperate deciduous forest system (Schmidt et al. 2015), while values of NREs in the grass rows were equal to those of a temperate grassland system (Keuter et al. 2013). The NREs of the tree rows in the Histosol and Anthrosol soils (Table A2) were within the range of NREs reported by Schmidt et al. (2015) for N (137−694 kg biomass m^{-2} $year^{-1}$/kg N m^{-2} $year^{-1}$) and Ca (3−34 kg biomass m^{-2} $year^{-1}$/kg Ca m^{-2}), and were higher for P (156−379 kg biomass m^{-2} $year^{-1}$/kg P m^{-2}), K (39−135 kg biomass m^{-2} $year^{-1}$/kg K m^{-2}) and Mg (43−200 kg biomass m^{-2} $year^{-1}$/ kg Mg m^{-2}). Additionally, the NRE for N of the tree rows in the Cambisol soil in 2016 (Table A2) was higher than those reported in Schmidt et al. (2015). The NRE values of grasses for N (Table A2) were within the range of those reported for a grassland in Germany with different management regimes (unfertilized control 89-113 kg biomass m^{-2} $year^{-1}$/kg N m^{-2} $year^{-1}$; Keuter et al. 2013).

The comparable NREs between the two management systems, grassland agroforestry and open grassland, propose that grassland agroforestry systems are relatively efficient compared to other land uses without sacrificing productivity.

4.2 Do fast growing trees reduce nutrient concentration in soil water and leaching losses in temperate grassland agroforestry?

4.2.1 Evaluation of the Soil Water Model

The modelled ratios of drainage/precipitation of the tree rows were comparable to those of other modelling studies, performed under similar climate and soil conditions, on short rotation coppices of similar age (3−6 years after establishment, Table 9). However, the drainage/precipitation ratios of the grass rows were either higher or lower than values modelled for several grassland sites (Table 9). The simulated study year of Wahren et al. (2015) had a slightly lower precipitation (500 mm yr^{-1}) than the study year for the respective Cambisol site (Table 4). Furthermore, Wahren et al. (2015) did not model the effect of tree shading on the water balance, probably resulting in a higher modelled annual evapotranspiration rate (411 mm yr^{-1}) compared to the modelled evapotranspiration at 1-m distance in the grass row (Table 4, Wahren et al. 2015). The higher drainage fluxes for grasslands: 484−507 mm yr^{-1} modelled by Hoeft et al. (2014) and 264−345 mm yr^{-1} modelled by Schmidt-Walter and Lamersdorf (2012), which resulted in higher drainage to precipitation ratios than ours, can be explained by the higher precipitation in the study years and sites of Hoeft et al. (2014, 1001−1083 mm) and Schmidt-Walter and Lamersdorf (2012, 651−662 mm). Since the modelled values for drainage flux were either comparable with literature values or deviations were explainable, it is assumed that the calculated water drainage fluxes were reliable. Hence, these fluxes were used for the calculation and interpretation of the nutrient leaching losses.

Table 9 Literature values of drainage/precipitation ratios from short rotation coppices (SRC) and grassland compared with data from the present study of grassland agroforestry (AF).

Study	Soil type	Management	Ratio water drainage/ precipitation
Wahren et al. (2015)	Cambisol	SRC with poplar trees	0.07
Wahren et al. (2015)	sandy soil	SRC with poplar trees	0.18
Schmidt-Walter and Lamersdorf (2012)	Anthrosol	SRC with willow trees	0.26−0.29
Wahren et al. (2015)	Cambisol	grassland	0.12
Hoeft et al. (2014)	haplic Cambisol	grassland	0.44−0.56
Schmidt-Walter and Lamersdorf (2012)	Cambisol	grassland	0.40−0.53
This study	Histosol	AF willow trees	0.18
This study	Anthrosol	AF willow trees	0.22
This study	Cambisol	AF willow trees	0.05
This study	Histosol	AF grassland	0.31−0.35
This study	Anthrosol	AF grassland	0.34−0.35
This study	Cambisol	AF grassland	0.25−0.36

The simulated shading effect of the tree rows on the grass rows in the agroforestry module substantially reduced photosynthetically active radiation for the grass rows, which in turn reduced grassland growth and correspondingly transpiration. The shading was therefore the main cause of higher water drainage fluxes in the grass rows closer to the tree rows compared to the grass at 4- and 7-m distance from the tree rows (Table 4). Presently, the agroforestry module does not include water or nutrient uptake by tree roots growing laterally from the tree rows into the grass rows. In an auxiliary study conducted at an alley cropping system with poplar trees and canola (crop row 48 m width), only 2−14 % of the total tree roots down to 1-m soil depth occurred in crop rows next to the tree rows (Hilgerdenaar et al. unpublished data). It is thus assumed that the omission of water uptake by lateral tree roots in the model did not have a major effect on the simulation results.

4.2.2 Effects of soil type on nutrient concentrations in soil water and leaching losses

The systematically different amounts of nutrient leaching losses between soil types can be related to their inherent soil properties (Table A3). Drained Histosol soils typically have very high N mineralization rates as the oxic conditions resulting from drainage lead to a very active microbial community, which uses the high organic material stocks as their substrate (Tiemeyer and Kahle 2014). This can explain the high soil NO_3^--N concentrations in Histosol soils, which in February and April 2017 were close to or even above the legal threshold value of 50 mg NO_3-N L^{-1} for ground water within the EU (EU 1998). NO_3^--N leaching losses of 3.1 to 4.2 g m^{-2} $year^{-1}$ have been reported from an intensively managed grassland ecosystem on a drained peatland in north-eastern Germany (Tiemeyer and Kahle 2014). These leaching losses were even higher than the leaching losses from the grassland rows of the Histosol soil (Table A3). Due to the low clay content of the Anthrosol, the effective cation exchange capacity (ECEC) was very low, especially in the subsoil (0.3−0.6-m depth; Table 2). This was probably the main reason why the Anthrosol soil had such high base cation losses (Table A3). The lower leaching losses of the Cambisol soil compared to Histosol and Anthrosol soils were partly related to soil properties (e.g. the Cambisol soil had the highest ECEC in 0.3−0.6-m depth, Table 2). However, management was influential since no fertilizer was applied at the Cambisol soil and the grass was harvested more frequently than on Histosol and Anthrosol soils (three times vs. one time). Both measures are known to reduce leaching losses (Hoeft et al. 2014). For all soil types P losses were below a critical threshold value for eutrophication (100 µg L^{-1}, Turner and Haygarth 1999).

4.2.3 Effects of tree rows on leaching losses and nutrient concentrations in soil water

Trees in the grassland agroforestry systems reduced leaching losses due to lower drainage flux under the tree rows (Table 4). These lower drainage fluxes together with higher evapotranspiration rates resulted in high concentration of nutrients but low nutrient losses in the tree rows. In order to further analyze how tree and grass rows affected nutrient concentrations in soil water, the ratio of nutrient/Na concentrations in soil water samples was used. Assuming that the Na^+ input through weathering does not differ within one soil type, and considering that Na^+ is not a critical nutrient for plants, Na^+ concentrations in soil water are affected either by deposition or by the water balance. Regarding the first reason, it is known that trees are typically more effective in collecting dust than grasses, which could lead to higher Na^+ deposition in the tree rows. However, under this supposition, one would expect significantly higher Na^+ concentrations at the site with the Cambisol soil where the canopy was larger than at the site with the Histosol and Anthrosol soils. This was not the case. The second potential reason is the water budget: more evapotranspiration will lead to higher Na^+ concentrations. For Histosol and Anthrosol soils, the higher evapotranspiration of the tree rows than the grass rows was also reflected in higher Na^+ concentrations in the tree rows ($p < 0.06$, Table 5). For Cambisol soil, no difference in Na^+ concentrations between tree and grass rows was seen. However, at the site with Cambisol soil, grass rows are considerably narrower (9-m width) compared to the site with Histosol and Anthrosol soils (48-m width). For this site I expect, that because of the minimal spacing between tree rows at the Cambisol soil site, tree roots grow underneath the entire grass row and trees dominate the system. Thus, the competition for water between trees and grasses is high. This is probably the main reason why no differences in Na^+ concentrations or nutrient/Na ratios for the Cambisol soil site were observed.

As the differences in Na^+ soil water concentrations were dominated by the soil water balance, any changes in the nutrient/Na ratios are the results of other processes. Such processes can either add nutrients relative to Na^+ (e.g. mineralization of nitrogen) or remove nutrients relative to Na^+ (e.g. nutrient uptake by vegetation or denitrification). It is known from an earlier study that net nitrification and net mineralization rates did not differ between tree and grass rows for Histosol and Anthrosol soils (Göbel et al. unpublished data). Furthermore, the amount of exchangeable bases was similar between tree and grass rows per soil type (Table 2). The increasing nutrient/Na ratio with distance to the tree rows for the Histosol and Anthrosol soils is thus interpreted as evidence for higher nutrient uptake by tree rows compared to grass rows. This is indicated by a significantly higher nutrient uptake to Na uptake ratios in the tree rows than in the grass rows (Table A4). Compared to grasses, the trees thus preferentially took up other nutrients in relation to Na, possibly since they required a higher amount of nutrients than the grasses.

Apart from water balance and nutrient uptake, trees can furthermore influence nutrient leaching losses by acting on microbial processes (Ribbons et al. 2018). This mechanism has been described for a poplar riparian buffer strip in southern England that had a better retention of NO_3^- in winter than a grassland riparian buffer strip (Haycock and Pinay 1993). It is assumed that this process also happened under the tree rows in the present study that showed lower NO_3^-/Na^+ ratios and thus indicated a removal of NO_3^- in the tree rows. The inclusion of trees into grassland could therefore potentially help to reduce NO_3^- losses also by acting on a microbial level.

4.3 How do gross rates of soil-N cycling in temperate grassland agroforestry and open grasslands differ?

Management (grassland agroforestry vs open grassland) or vegetation type (tree or grass) did not affect soil-N cycling, thus opposing the hypothesis that gross rates of soil-N cycling are higher in the tree rows than in the grass rows or open grassland. Instead, N-cycling rates and microbial biomass were rather affected by soil types as seen by the split-up into soil types in Fig 8. Previous studies have found contradicting results of the effect of land-use management on N-cycling rates. Whereas some studies found no effect of different management on N-cycling (Bedard-Haughn et al. 2006, Ribbons et al. 2018), Banerjee et al. (2016) found stronger effects of different land-uses than soil types on bacterial community size and richness. One reason why no effects of management systems were detected in the present study could be the relatively young age (5−7 years) of the investigated systems in regard to a general cultivation time of alley cropping agroforestry of about 30 years and older (N. Lamersdorf, pers. communication). Previous studies have found no change in soil properties in short time-spans, but only after at least ten years of a system's establishment (Oelbermann and Voroney 2007, Pardon et al. 2017). A first hint on changing soil properties in the investigated systems over time could be the higher fungi to bacteria ratio in the tree rows than in grass rows and open grassland (Beule et al. unpublished data). This shift in microbial abundance is explained by the more recalcitrant litter of the trees, whilst grassland soil organic matter is more mineralizable (Booth et al. 2005). However, there was no difference yet seen in the soil C:N ratio of the 0.3-m depth or the microbial C:N ratio on the study sites (Tables 1 and 8). In consequence, opposing the hypothesis, microbial biomass size did not increase under tree rows and thus gross N mineralization rates did not increase either.

Even though vegetation type only played a minor role in influencing N-cycling, slight feedbacks were seen for the Cambisol soil. Plant composition of open grassland and the grass rows in agroforestry on the Cambisol soil might have not been exactly the same since the open grassland plots were three years younger than the grass rows in the agroforestry system and the amount of clover is known to decline with increasing age (J. Isselstein, pers. communication). Furthermore, clover is a heliophilous plant which might have led to a further decrease of clover in the agroforestry grass rows exposed to shading from the trees (Ehret et al. 2015). Shade probably was also the main cause for a significant higher aboveground biomass production in open grassland compared to 1-m distance of the agroforestry system for this site (Göbel et al. unpublished data). It is assumed that this higher biomass production led to increased return of available organic matter to the soil, and in turn fostered microbial activity (Corre et al. 2002). To maintain this high amount of microbial biomass, open grassland at the Cambisol site consequently also had a higher NH_4^+ immobilization than the grass rows in agroforestry (Tables 7 and 8).

Microbial biomass was a strong driver for gross N mineralization (Fig 8 a) and itself was driven by soil fertility indicators such as total N content, C:N ratio and ECEC (Fig 8 b-d). The soils of the study sites are highly fertile (i.e. high total N, pH, ECEC, base saturation, Table 2). In such fertile soils, any possible competition for available nutrients between vegetation (trees or grass) and microbial biomass is likely to be low (Bardgett et al. 2003, Schmidt et al. 2016). Therefore, establishment of trees as alley cropping agroforestry systems on previous grassland on fertile soils may not impose competition for nutrient resource acquisition. Gross N mineralization rates of the present study were comparable with previous studies in temperate forests and grasslands. Tree rows on all soil types showed similar gross N mineralization rates to those measured in a temperate beech forest on a Dystric Cambisol (206 ± 42 mg N $m^{-2}day^{-1}$, Corre et al. 2003; Table 7). Gross N mineralization rates of the grass rows and open grassland (Table 7) were slightly lower than data measured by Hoeft et al. (2014) for an unfertilized grassland on a Haplic Cambisol (824 ± 170 mg N m^{-2} day^{-1}) even though MBN was in range with values by Hoeft et al. (2014) of 6104 ± 226 mg N m^{-2} (Table 8). This further undermines that grassland agroforestry can be a sustainable alternative to open grassland in terms of N availability.

The average length of time a nutrient cycles in a certain pool is known as the mean residence time. A faster turnover rate thus goes along with a lower mean residence time. The higher turnover times of NO_3^- than NH_4^+ in Histosol and Anthrosol soils increase the risk of leaching losses when precipitation occurs during this time, as NO_3^- is more easily leached from the soil than NH_4^+ (Table 8). These findings are supported by an auxiliary study that reported low NO_3^- leaching losses from Cambisol soil and higher NO_3^- leaching losses from the Histosol and Anthrosol soils during April 2016–April 2017 (Göbel et al. unpublished data). However, the investigated gross N cycling rates can only be understood as a snapshot in time and especially the Histosol soil has a thick organic layer of which only the top 0.05 m were investigated. Since gross nitrogen transformations underlie a seasonal variability depending on temperature and soil moisture, long-term measurements of soil-N cycling over different seasons should be considered in the future (Wang et al. 2016). At the time of sampling, though, (April 2017 for Histosol and Anthrosol soils, July 2015 for Cambisol soil), it is concluded that both management systems, grassland agroforestry and open grassland, were sustainable in their gross N-cycling.

5 Synthesis

5.1 Key findings

The key findings of the three studies allow more general conclusions whether grassland agroforestry can be a sustainable alternative management system to open grassland in Germany.

5.1.1 Can temperate grassland agroforestry be a sustainable alternative to open grassland in terms of soil nutrient availability and nutrient response efficiency?

This study shows that grassland agroforestry can be a sustainable alternative system to open grassland without sacrificing productivity in terms of nutrient availability and nutrient response efficiency. The underlying hypothesis was that based on an increase in productivity in the grassland agroforestry system due to the trees, nutrient response efficiency would be higher in grassland agroforestry compared to open grassland.

Water and nutrient availability in the top 0.05 m were mostly equal among the two management systems in the two study years. Regarding biomass and NRE, the grassland agroforestry systems behaved differently in the two study years. In 2016, biomass production was equal between the systems. This resulted in generally comparable NRE of the systems (area-weighted values of the tree and grass rows). An explanation could be that both systems exhibited optimum or saturated levels of soil nutrients so that the hypothesized higher NREs of grassland agroforestry did not occur. Higher NREs for Ca and Mg in open grassland than grassland agroforestry in the Cambisol soil provided an exception. This finding was attributed to the low biomass production of the grass row in 1-m distance from the trees which could not be outweighed by higher production of the grass row further away from the trees in this narrow grass row of only 9-m width. Therefore, when implementing an agroforestry system, consideration of the width of alley rows is important such that any possible decrease in biomass production closer to the trees can be compensated by the areas farther away from the trees.

In 2017, biomass production of the grassland agroforestry systems was significantly higher than that of open grassland resulting in higher NREs of the grassland agroforestry system. This suggests that grassland agroforestry systems are getting more efficient with time (i.e. when the trees mature).

5.1.2 Do fast growing trees reduce nutrient concentration in soil water and leaching losses in temperate grassland agroforestry?

This study shows that fast growing trees can reduce leaching losses in temperate grassland systems – under certain conditions – and thus may contribute to better groundwater quality. In this study it was hypothesized that 1) nutrient leaching losses will be smaller in the tree rows than in the grass rows of the agroforestry systems, and 2) in the grass rows, nutrient losses will increase with distance from the tree rows.

The results support the first hypothesis that is: leaching will be smaller in the tree rows than in the grass rows. This is based on the reduction of drainage under the tree rows compared to grass rows due to higher evapotranspiration of trees. Furthermore, trees positively influenced nutrient losses by preferential nutrient uptake and possibly due to increased microbial processes such as denitrification under tree rows. The effect was higher closer to the tree rows (e.g. gradient of nutrient/Na ratios for Histosol and Anthrosol soils), thus undermining the second hypothesis that nutrient losses will increase in the grass rows with distance from the tree rows. For the Cambisol soil, however, with smaller grass rows (9-m width), the tree rows were so close that tree effects (i.e. competition for water) dominated the whole grass row. This suggests that trees have to be implemented in a certain way based on the site conditions to achieve optimal results for better groundwater quality.

5.1.3 How do gross rates of soil-N cycling in temperate grassland agroforestry and open grasslands differ?

This study shows that grassland agroforestry can be equally efficient in terms of soil N availability and soil-N cycling compared to open grassland and can thus be a sustainable alternative management system. It was hypothesized that if the short rotation trees increase microbial biomass size as a consequence of increase in organic matter input from litter fall and root turnover, gross rates of soil-N cycling will be higher in the tree rows than in the grass rows or open grassland.

In the present study, not the different land-use (grassland agroforestry vs. open grassland) or vegetation type (tree or grass) but the different soil types (fertility gradient) were driving the soil N-cycling. This could possibly be due to the young age (5–7 years) of the investigated systems where tree rows still had a comparable C:N ratio compared to grass rows. Consequentially, microbial biomass and thus also gross N mineralization rates did not increase as hypothesized. However, as N-cycling is highly variable, the study only represents a snapshot in time.

5.2 Implementation of agroforestry

While grassland agroforestry can be managed equally profitably (in terms of biomass production) as open grassland, it also further improves important soil functions. As described by Graves et al. (2007) agriculture and forestry grown together result in a higher value of ecosystem services than if grown separate. It was also shown that nutrient cycling is efficient in these systems and – at least in these young systems (5–8 years old) – no competition for nutrients or water seems to exist. Even though advantages of agroforestry systems in temperate areas are being rediscovered, there is only a poor implementation of such systems in Germany. Whereas Mediterranean countries such as Portugal or Greece cultivate agroforestry systems on over 30 % of their utilized agricultural area, Germany is ranging at the lower end of European countries with only 1.6 % (den Herder et al. 2017). In Europe about 90 % of the total area covered with agroforestry systems are linked to silvopastoral systems. These silvopastoral systems cover around 12 % of the total grassland area in Europe, mostly located in southern countries (Mosquera-Losada et al. 2018)

However, in Europe several reasons are currently hindering farmers to implement agroforestry systems on their land. These are e.g. high establishment costs (Nerlich et al. 2013), management complexity (Wolz et al. 2017), administrative burden (Tsonkova et al. 2018), lack of information (Graves et al. 2009), lack of positive examples (Reeg 2011) and lack of financial incentives (Smith et al. 2012, Langenberg et al. 2018). Furthermore, a long-term perspective is necessary for planning and establishing agroforestry systems. The main problem, however, is uncertainty on the legislative level (Borremans et al. 2016, Tsonkova et al. 2018). Agriculture in Europe and thus also Germany strongly depends on subsidiaries of the Common Agricultural Policy (CAP) of the EU which encompasses about 40 % of the EU budget. The CAP is based onto two pillars (EU directive 1311/2013): The first pillar is used for direct payments based on the area a farmer is cultivating; the second pillar is used for special measures fostering rural development. Agroforestry is currently implemented both in the first (EU directive 1307/2013) as well as in the second pillar (EU directive 1305/2013). However, in regard to the first pillar, alley cropping systems cannot be accounted for as a whole system in Germany as there currently exists no land use code for agroforestry in the German system. This code is needed for administration by the Integrated Administration and Control System (IACS; Tsonkova et al. 2018). Furthermore, the minimum area that can be registered in IACS is 0.3 ha, which thus excludes establishment of small-scale alley cropping systems (Böhm et al. 2017). Additionally, if there was an accepted definition, agroforestry could also be considered as an Ecological Focus Area in the first pillar (part of the EU's Greening program) (Tsonkova et al. 2018). For the second pillar member states of the EU based on country-specific needs (e.g. pedoclimatic and environmental conditions) can decide which of the suggested measures and programs by the EU to implement. In Germany, due to its federal structure, this decision is again divided into specific regional programs. Currently no financial support is granted for the establishment of an agroforestry system in Germany, which could have been provided by the EU directive 1305 (Tsonkova et al. 2018). The reform of the EU's Common Agricultural Policy, running from 2021–2017, could be a chance to foster agroforestry implementation in Germany and Europe.

5.3 Conclusion

Generally, a stronger impact on ecosystem services of silovarable systems (i.e. trees and cropland) compared to monoculture cropland than silvopastoral systems (i.e. trees and grassland) compared to open grassland has been found (Torralba et al. 2016). Nevertheless, since competition and prices for arable land in Germany are very high (Destatis 2018), implementation of trees onto existing grassland could be a chance to fill the "wood gap" mentioned in the introduction, while at the same time providing environmental services and producing feedstuff. As grassland is often located at less fertile sites, it is often cheaper than cropland (J. Isselstein, pers. communication) and at risk of being abandoned since traditional grassland management is often not compatible with conditions required for highly productive grassland (Isselstein et al. 2005). Grassland agroforestry could thus also help to retain grassland which is an important land-use under nature conservation aspects as well. Grassland agroforestry systems can therefore be one measure to solve the "land-use trilemma".

Central aim of the SIGNAL project is to evaluate whether and under which site conditions agroforestry in Germany can be a land use alternative that is ecologically, economically and socially more sustainable than conventional agriculture. This thesis shows that temperate grassland agroforestry can be a sustainable alternative management system to open grassland in terms of several ecological indices. However, further aspects should be investigated. Agroforestry systems are dynamic systems with changing ecological interactions over time. They can experience a complex series of inter- and intra-specific interactions due to modification and utilization of light, water and nutrients differing with tree growth (Jose et al. 2004). This is particularly true for soil-N cycling that underlies a seasonal variability (e.g. Wang et al. 2016). Previous studies have furthermore shown that NRE differs with time (Keuter et al. 2013). Therefore, long-term evaluations with a sufficient replicate number (more than three plots) and open grassland plots with exactly the same conditions (e.g. ground water fluxes, age of grass swards) are required to assess the sustainability of the short-term rotations (4–5 years) for trees grown for bioenergy production. Future study sites should focus on an intensive grassland management (several cuts throughout the vegetation period, periodic fertilization), that is more common in agricultural practice than the management of the study sites in this thesis. The longer-term evaluations are also needed to assess economic benefits of agroforestry in order to strengthen the basis for further incorporating agroforestry into a European and German policy framework.

Economic success, and thus implementation of agroforestry systems, strongly depends on the future decision of the EU how to implement agroforestry systems (especially into existing grassland) into subsidiary payments for the next phase of the CAP of 2021–2027. So far, no beneficial reward of ecological benefits from agroforestry systems is considered in the subsidiaries. Scientific research such as the SIGNAL project can provide scientific data and knowledge to foster the implementation of agroforestry in politics on EU and national level and consequently in practical farming. Based on the result of this thesis, adaptions in the Common Agricultural Policy of the European Union and the German agricultural policy, which currently prevents farmers from implementing trees on existing grassland, are strongly proposed, to foster the establishment of grassland agroforestry systems.

Appendix

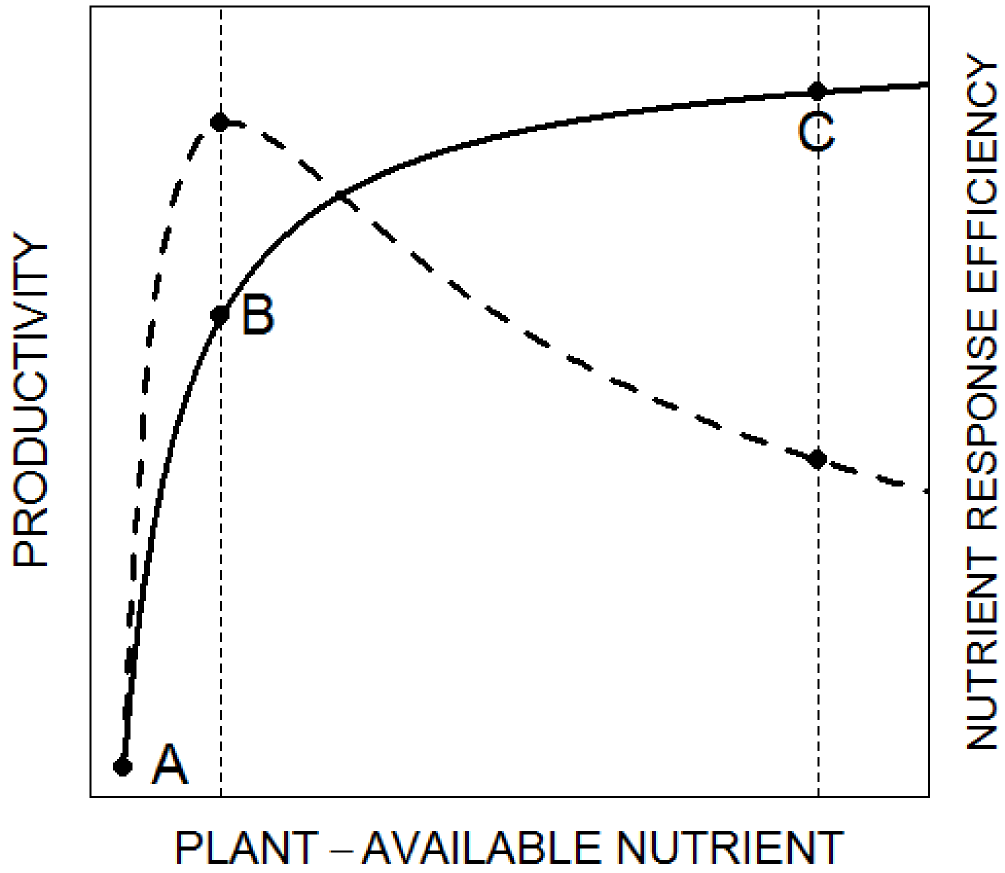


Fig A1 Growth curve (solid line) and nutrient response efficiency (NRE) curve (dashed line) in the case of ideal dependency of productivity on a plant-available nutrient in the soil. (A) Zero productivity at minimum soil nutrient level; (B) productivity at optimum NRE; (C) maximum productivity at nutrient saturation (printed with permission from Schmidt et al. (2015)).

Table A10 Plant-available nutrients and water-filled pore space (WFPS) in soils of grassland agroforestry (AF) and adjacent open grasslands on three soil types in central Germany, measured in the top 0.05 m in March−October 2016.

Soil type	Management system	Sampling location	Plant-available N ($g N m^{-2}$ year)	Plant-available P ($g P m^{-2}$)	Exchangeable K ($g K m^{-2}$)	Exchangeable Ca ($g Ca m^{-2}$)	Exchangeable Mg ($g Mg m^{-2}$)	WFPS (%)
Histosol soil	AF	tree row	6.5 ± 1.4 a	4.4 ± 0.5 a	1.8 ± 0.4 b	71 ± 11 a	2.7 ± 0.2 b	36 ± 7 b
		1 m	14.5 ± 2.9 a	4.7 ± 0.7 a	2.4 ± 0.3 b	101 ± 10 a	4.4 ± 0.7 b	42 ± 8 ab
		4 m	15.1 ± 3.8 a	4.5 ± 0.2 a	4.0 ± 0.4 ab	94 ± 6 a	4.3 ± 0.5 b	39 ± 4 ab
		7 m	11.2 ± 3.4 a	4.8 ± 0.3 a	5.5 ± 1.2 a	116 ± 16 a	6.6 ± 0.6 a	48 ± 8 a
	Open	Open	12.7 ± 3.3 a	3.6 ± 0.2 a	3.1 ± 0.5 ab	109 ± 18 a	3.5 ± 0.1 b	37 ± 6 ab
Anthrosol soil	AF	tree row	6.5 ± 3.4 a	3.7 ± 0.1 b	1.7 ± 0.2 a	44 ± 3 a	2.0 ± 0.2 a	29 ± 1 b
		1 m	3.4 ± 0.3 a	4.4 ± 0.4 a	3.2 ± 0.5 a	60 ± 7 a	3.2 ± 0.3 a	34 ± 1 a
		4 m	3.5 ± 1.2 a	4.5 ± 0.4 a	2.6 ± 0.3 a	63 ± 14 a	3.2 ± 0.5 a	39 ± 4 a
		7 m	4.6 ± 1.5 a	4.8 ± 0.4 a	2.6 ± 0.2 a	63 ± 6 a	3.0 ± 0.3 a	39 ± 2 a
	Open	Open	4.8 ± 0.8 a	4.2 ± 0.1 ab	2.2 ± 0.3 a	56 ± 14 a	2.5 ± 0.6 a	33 ± 2 ab
Cambisol soil	AF	tree row	2.5 ± 0.6 a	7.2 ± 0.7 ab	17.0 ± 0.8 a	91 ± 11 a[1]	6.7 ± 1.3 a	73 ± 5 a
		1 m	1.7 ± 0.8 a	5.2 ± 0.4 c	8.2 ± 0.4 b	83 ± 8 a	5.3 ± 1.0 a	80 ± 5 a
		4 m	5.1 ± 2.2 a	6.2 ± 1.1 bc	8.5 ± 0.3 b	138 ± 52 a	5.5 ± 0.8 a	74 ± 3 a
	Open	Open	5.1 ± 1.8 a	7.9 ± 0.6 a	14.0 ± 1.9 a	90 ± 17 a	4.5 ± 0.6 a	60 ± 3 a

Means ± standard errors (n = 3 for Histosols and Anthrosols, n = 4 for Cambisols) within each soil type followed by a different letter indicate significant differences among sampling locations within grassland agroforestry (i.e. tree rows and various distances within the grass rows) and open grasslands (LME model at $p \leq 0.05$ for the repeatedly measured plant-available N, P and WFPS; one-way ANOVA with Fisher's LSD test at $p \leq 0.05$ or Kruskal-Wallis H test with multiple comparison extension at $p \leq 0.05$ for the one-time measured exchangeable K, Ca and Mg for Histosol and Anthrosol soils; ANCOVA with Fisher's LSD test at $p \leq 0.05$ for the one-time measured exchangeable K, Ca and Mg for Cambisol soil)

[1] Differences among sampling locations cannot be tested using ANCOVA because of multicollinearity, i.e., the effect of the different distances to the tree rows cannot be statistically distinguished from that of the clay content

Table A2 Nutrient response efficiencies (NRE) and aboveground biomass production of grass and trees (wood + leaf litter) of grassland agroforestry (AF) and adjacent open grasslands on three soil types in central Germany in 2016 and 2017.

Soil type	Management system	Sampling location	NRE N (kg biomass m^{-2} year^{-1}/ kg N m^{-2} year^{-1})	NRE P	NRE K	NRE Ca	NRE Mg	Harvestable aboveground biomass[1] (kg m^{-2} year^{-1})
				(kg biomass m^{-2} year^{-1}/ kg available nutrient m^{-2})				
2016								
Histosol soil	AF							0.7 ± 0.1 A
		tree row	161 ± 5 a	228 ± 27 a	595 ± 117 a	15 ± 4 a	388 ± 77 a	1.0 ± 0.2 a
		1 m	26 ± 5 b	75 ± 3 c	159 ± 42 ab	4 ± 1 a	88 ± 22 b	0.4 ± 0.1 b
		4 m	44 ± 9 b	133 ± 14 bc	152 ± 8 ab	6 ± 1 a	146 ± 27 b	0.6 ± 0.1 ab
		7 m	54 ± 20 b	108 ± 21 bc	94 ± 4 b	4 ± 0 a	76 ± 10 b	0.5 ± 0.1 ab
	Open		55 ± 21 b	160 ± 19 ab	195 ± 45 ab	5 ± 1 a	161 ± 17 b	0.6 ± 0.0 A ab
Anthrosol soil	AF							0.5 ± 0.0 A
		tree row	190 ± 65 a	221 ± 12 a	480 ± 57 a	18 ± 1 a	403 ± 32 a	0.8 ± 0.0 a
		1 m	62 ± 3 a	49 ± 9 b	71 ± 14 b	4 ± 1 b	69 ± 10 b	0.2 ± 0.0 b
		4 m	175 ± 60 a	106 ± 25 b	181 ± 33 b	8 ± 2 b	152 ± 37 b	0.5 ± 0.1 b
		7 m	112 ± 24 a	96 ± 19 b	173 ± 23 b	7 ± 2 b	152 ± 18 b	0.5 ± 0.1 b
	Open		108 ± 30 a	114 ± 12 b	225 ± 39 b	10 ± 3 ab	214 ± 65 ab	0.5 ± 0.0 A b
Cambisol soil	AF							0.9 ± 0.1 B
		tree row	783 ± 465 a	151 ± 32 a	64 ± 14 a	12 ± 3 a	174 ± 47 ab	1.1 ± 0.3 ab
		1 m	3617 ± 3262 a	114 ± 15 a	74 ± 13 a	7 ± 2 a	128 ± 34 b	0.6 ± 0.1 b
		4 m	342 ± 177 a	163 ± 41 a	106 ± 10 a	9 ± 2 a	177 ± 37 ab	0.9 ± 0.1 ab
	Open		419 ± 156 a	168 ± 6 a	100 ± 15 a	16 ± 2 a	309 ± 39 a	1.3 ± 0.1 A a
2017								
Histosol soil	AF							0.9 ± 0.2 A
		tree row		416 ± 56 a	1073 ± 184 a	28 ± 7 a	706 ± 144 a	1.9 ± 0.4 a
		1 m		67 ± 8 b	132 ± 19 b	3 ± 0 b	72 ± 9 b	0.3 ± 0.1 b
		4 m	-	104 ± 33 b	119 ± 34 b	5 ± 1 b	109 ± 33 b	0.5 ± 0.1 b
		7 m		82 ± 11 b	74 ± 7 b	3 ± 0 b	59 ± 6 b	0.4 ± 0.1 b
	Open			91 ± 15 b	108 ± 23 b	3 ± 0 b	91 ± 11 b	0.3 ± 0.1 b B
Anthrosol soil	AF							0.8 ± 0.1 A
		tree row		445 ± 21 a	966 ± 115 a	37 ± 2 a	811 ± 72 a	1.6 ± 0.1 a
		1 m		60 ± 12 b	88 ± 21 b	4 ± 1 b	86 ± 20 b	0.3 ± 0.0 b
		4 m	-	63 ± 5 b	109 ± 6 b	5 ± 1 b	91 ± 12 b	0.3 ± 0.1 b
		7 m		72 ± 17 b	141 ± 41 b	5 ± 1 b	123 ± 38 b	0.4 ± 0.0 b
	Open			98 ± 11 b	196 ± 39 b	8 ± 2 b	169 ± 23 b	0.4 ± 0.1 b B
Cambisol soil	AF							1.2 ± 0.04 A
		tree row						1.9 ± 0.02 a
		1 m			-			0.6 ± 0.06 c
		4 m						0.8 ± 0.06 b
	Open							1.0 ± 0.03 b B

Means ± standard errors (n = 3 for Histosol and Anthrosol soils, n = 4 for Cambisol soil) within each soil type followed by a different lowercase letter indicate significant differences among sampling locations within grassland agroforestry (i.e. tree rows and various distances within the grass rows) and open grasslands per year (one-way ANOVA with Fisher's LSD test at $p \leq 0.05$ or Kruskal-Wallis H test with multiple comparison extension at $p \leq 0.05$ for Histosol and Anthrosol soils; ANCOVA with Fisher's LSD test at $p \leq 0.05$ for Cambisol soil). Means within each soil type followed by a different uppercase letter indicate significant differences between the two management systems (Independent T test or Mann-Whitney-Wilcoxon test at $p \leq 0.05$; ANCOVA with Fisher's LSD test at $p \leq 0.05$ for Cambisol soil)

[1] In Histosol and Anthrosol soils, the trees were planted in 2008, cut for the first time in Jan. 2016 and were in the first year of the second rotation during the study year 2016 (Swieter et al. unpublished data). In the Cambisol soil, the trees were planted in 2011, cut for the first time in Jan. 2015 and were in the second year of the second rotation during the study year 2016 (Malec et al. unpublished data)

Table A3 Monthly nutrient leaching fluxes from 0.6-m depth under tree rows and several distances to the tree rows within grass rows in temperate grassland agroforestry on three soil types in central Germany from the study period April 2016–April 2017.

Leaching component (mg nutrient m^{-2} $month^{-1}$)	tree row	1 m	4 m	7 m
	Histosol soil			
Ammonium	1.88 ± 0.58 a	2.42 ± 0.77 a	3.72 ± 1.21 a	2.66 ± 0.88 a
Nitrate	94 ± 43 a	190 ± 109 a	188 ± 94 a	239 ± 156 a
Dissolved organic N	36 ± 11 a	76 ± 37 a	74 ± 32 a	55 ± 20 a
Total dissolved N	132 ± 49 a	268 ± 145 a	266 ± 126 a	297 ± 173 a
Calcium	751 ± 266 a	1585 ± 587 a	882 ± 302 a	610 ± 221 a
Magnesium	66 ± 25 a	152 ± 57 a	91 ± 32 a	74 ± 26 a
Potassium	12 ± 5 a	12 ± 4 a	7 ± 2 a	12 ± 4 a
Sodium	264 ± 91 a*	287 ± 104 a*	44 ± 14 ab	48 ± 16 b*
Phosphate	0.03 ± 0.02 a	0.06 ± 0.04 a	0.05 ± 0.04 a	0.04 ± 0.03 a
$P_{organic}$	0.11 ± 0.05 b	0.19 ± 0.08 ab	0.18 ± 0.07 ab	0.23 ± 0.08 a
	Anthrosol soil			
Ammonium	1.96 ± 0.79 a	1.90 ± 0.66 a	2.26 ± 0.70 a	1.91 ± 0.77 a
Nitrate	20 ± 7 a	50 ± 21 a	40 ± 13 a	35 ± 16 a
Dissolved organic N	13 ± 4 a	17 ± 7 a	17 ± 5 a	10 ± 5 a
Total dissolved N	35 ± 10 a	69 ± 27 a	59 ± 17 a	47 ± 21 a
Calcium	348 ± 132 a	305 ± 110 a	217 ± 62 a	139 ± 55 a
Magnesium	35 ± 13 a*	33 ± 12 a*	20 ± 6 ab	15 ± 6 b*
Potassium	6 ± 2 a	3 ± 1 a	4 ± 1 a	6 ± 3 a
Sodium	158 ± 59 a	73 ± 26 ab	56 ± 15 b	44 ± 19 b
Phosphate	0.04 ± 0.02 a	0.02 ± 0.01 a	0.04 ± 0.03 a	0.03 ± 0.02 a
$P_{organic}$	0.09 ± 0.06 a	0.06 ± 0.02 a	0.07 ± 0.03 a	0.06 ± 0.03 a
	Cambisol soil			
Ammonium	0.17 ± 0.04 b	0.28 ± 0.08 a	0.26 ± 0.07 ab	
Nitrate	0.4 ± 0.1 a	0.9 ± 0.2 a	6.6 ± 3.2 a	
Dissolved organic N	0.4 ± 0.0 a	2.4 ± 1.2 a	1.0 ± 0.3 a	
Total dissolved N	1.0 ± 0.1 a	3.6 ± 1.4 a	7.9 ± 3.3 a	
Calcium	108 ± 37 a	288 ± 66 a	114 ± 32 a	
Magnesium	59 ± 23 a	80 ± 16 a	42 ± 12 a	
Potassium	8 ± 2 b	35 ± 10 ab	26 ± 7 a	
Sodium	16 ± 5 b	43 ± 9 a	22 ± 5 a	
Phosphate	0.03 ± 0.01 b	0.24 ± 0.07 a	0.11 ± 0.03 ab	
$P_{organic}$	0.03 ± 0.01 a	0.04 ± 0.02 a	0.00 ± 0.00 a	

Means ± standard errors (n = 3 for Histosol and Anthrosol soils, n = 4 for Cambisol soil) followed by a different letter indicate significant differences between sampling locations within each soil type (LME model or generalized mixed models with Fisher's LSD test at $p \leq 0.05$)

* $p < 0.1$

Table A4 Plant nutrient uptake from tree rows and several distances to it within grass rows in temperate grassland agroforestry on three soil types in central Germany.

Soil type	Sampling location	N uptake	P uptake	K uptake	Ca uptake	Mg uptake	Na uptake
		(g nutrient m^{-2} $year^{-1}$)					
Histosol soil	tree row	10.4 ± 1.7 a	0.9 ± 0.2 a	2.4 ± 0.5 b	6.4 ± 0.8 a	0.9 ± 0.1 a	0.14 ± 0.02 b*
	1 m	6.5 ± 0.8 a	0.8 ± 0.2 a	4.5 ± 0.6 ab	1.2 ± 0.3 b	0.5 ± 0.0 b	1.24 ± 0.38 ab
	4 m	10.8 ± 0.4 a	1.2 ± 0.1 a	8.0 ± 1.1 a	2.2 ± 0.3 b	0.9 ± 0.0 a	1.49 ± 0.35 a
	7 m	8.9 ± 1.9 a	1.0 ± 0.2 a	6.6 ± 1.5 ab	1.9 ± 0.4 b	0.8 ± 0.0 ab	1.12 ± 0.20 ab
Anthrosol soil	tree row	7.6 ± 0.2 a*	0.7 ± 0.0 a	2.0 ±0.0 b	5.1 ± 0.5 a	0.6 ± 0.1 a	0.09 ± 0.00 b
	1 m	3.6 ± 0.3 b	0.6 ± 0.1 a	4.2 ± 0.5 ab	0.6 ± 0.0 b	0.3 ± 0.0 a	0.05 ± 0.03 b
	4 m	5.8 ± 1.4 ab	1.0 ± 0.2 a	6.6 ± 1.5 ab	1.3 ± 0.3 b	0.6 ± 0.0 a	0.69 ± 0.09 a
	7 m	6.2 ± 1.1 ab	1.0 ±0.2 a	7.2 ± 1.4 a	1.3 ± 0.2 b	0.6 ± 0.0 a	0.30 ± 0.11 b
Cambisol soil	tree row	6.2 ± 1.3 b	1.1 ± 0.2 c	3.4 ± 0.7 c	5.2 ± 1.0 ab*	0.5 ± 0.1 b	0.1 ± 0.0 c
	1 m	11.6 ± 2.0 b	1.9 ± 0.2 b	16.8 ± 2.5 b	4.3 ± 0.9 b	0.9 ± 0.1 ab	0.4 ± 0.0 b
	4 m	21.2 ± 2.9 a	2.8 ± 0.2 a	25.3 ± 3.0 a	8.1 ± 1.1 a	1.5 ± 0.2 a	0.6 ± 0.0 a

Means ± standard errors (n=3 for Histosol and Anthrosol soils, n=4 for Cambisol soil) followed by a different letter indicate significant differences between sampling locations within each soil type (one-way ANOVA with Fisher's LSD test or Kruskal-Wallis H test with multiple comparison extension at $p \leq 0.05$)

* $p < 0.1$

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Contributions

The basis for this thesis were three manuscripts written within project 1.1. of the SIGNAL initiative. Project 1.1. was conceptualized by Prof. Dr. Edzo Veldkamp, Dr. Marife Corre and Prof. Dr. Norbert Lamersdorf. Several people contributed to these three manuscripts, in the following they are named in the order of their contributions. Field and laboratory work was carried out by Leonie Göbel and Dr. Marcus Schmidt. Data for soil-N cycling (study three) from Reiffenhausen were obtained from Lin Chen. Further data, such as biomass data, were taken from project partners within SIGNAL (see the respective references within the thesis). Dr. Florian Heinlein, Prof. Dr. Eckart Priesack and Leonie Göbel developed the agroforestry module in Expert-N which Leonie Göbel used to model drainage fluxes. Leonie Göbel conducted the statistical analyses with assistance from Marcus Schmidt and Marife Corre. The original drafts of all three studies were written by Leonie Göbel. Marife Corre, Edzo Veldkamp, Marcus Schmidt, Norbert Lamersdorf, and Johannes Isselstein reviewed and edited the first study (NRE), Edzo Veldkamp, Marife Corre, Marcus Schmidt, and Florian Heinlein reviewed and edited the second study (leaching losses). Leonie Göbel wrote the synthesis of this thesis.

Declaration of originality and certificate of authorship

I, Leonie Göbel, hereby declare that I am the sole author of this dissertation entitled "Nutrient Response Efficiencies, Leaching Losses and Soil-N cycling in Temperate Grassland Agroforestry and Open Grassland Management Systems", and that all references and data sources have been appropriately acknowledged. I furthermore declare that this work had not been submitted elsewhere in any form as part of another dissertation procedure.

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Talks and poster presentations

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Göbel L, Schmidt M, Veldkamp E, Corre M (2017): Nutrient response efficiency in temperate grassland agroforestry systems. IUFRO, Freiburg i. Brg. *Oral presentation* – 09/2017.

Swieter A, Langhof M, Greef JM, Schmidt M, **Göbel L** (2017): Einfluss der Baumstreifen auf den mineralischen Stickstoff-Verlauf im Oberboden in einem Alley Cropping-Agroforstsytem. DBG Jahrestagung, Göttingen. *Poster presentation* – 09/2017.

Göbel L, Schmidt M, Veldkamp E, Lamersdorf N, Corre M (2016): Nährstoffnutzungs- und Nährstoffretentionseffizienzen in Agroforstsystemen. 5. Agroforstforum, Senftenberg. *Poster presentation* – 11/2016.

Göbel L, Schmidt M, Lamersdorf N, Veldkamp E, Corre M (2016): Soil nitrogen cycling in a grassland alley cropping system versus conventional grassland. BONARES Status-Seminar, Leipzig. *Poster presentation* – 11/2016.

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