



Decoupling of subsoil carbon and nitrogen dynamics after long-term crop rotation and fertilization



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ABSTRACT

Enhancing global soil organic carbon storage by 4 per mille (‰) per annum would be enough to halt current net greenhouse gas emissions, but this goal seems lofty for conventional agriculture, which frequently results in soil organic carbon and nitrogen losses. Replacing mineral nitrogen with organic nitrogen sources may benefit soil carbon and nitrogen cycling in agricultural soils, but long-term effects are yet to be clearly demonstrated, especially in soils of high natural fertility. Here we report the effects of 34 years of legumes (crimson clover, fava beans) and non-legumes (maize) in rotation combined with different fertilization regimes (no fertilization, PK fertilization, NPK fertilization) on soil carbon and nitrogen storage throughout the uppermost meter of the soil profile. Fava beans did not enhance profile carbon storage. However, fava beans induced positive effects on subsoil nitrogen cycling, with lower subsoil nitrogen densities indicating lower nitrogen leaching potential. Incorporating a clover green mulch every 4 years enhanced organic carbon storage by an average of 4.1‰ per annum down the full meter of soil compared with a conventional maize rotation, but only combined with phosphorus and potassium fertilization. The enhancement was detected below the plough-horizon, indicating that merely sampling topsoil is insufficient to assess soil carbon dynamics in these arable soils. In contrast, maize contributed only a small portion to SOC, with subsoil C contributions negligible. These results indicate that a careful combination of long-term, site-adapted crop and fertilization management strategies can help enhance SOC storage in naturally fertile soils without apparent C deficit.

1. Introduction

Loss of soil organic carbon (SOC) is a threat to earth systems, as SOC fulfils a variety of ecosystem functions, including but not limited to boosting soil fertility, regulating climate and buffering soil systems against adverse climate impacts (Paustian et al., 2016). For decades, scientists across the globe have advocated enhancing SOC storage, and recently a global target of annual SOC enhancement of 4‰ has been calculated as being sufficient to offset current anthropogenic greenhouse gas emissions (Lima Paris Agreement Agenda, 2015; Lal, 2016).

One proposed strategy for climate-smart soil management is to reduce the application of mineral nitrogen fertilizer (N_{\min}) to soils (Paustian et al., 2016). The conversion of atmospheric N_2 into N_{\min} during the Haber-Bosch process was a key driver of the Green Revolution, which has fed a large portion of the world (Smil, 1999). However, this process is associated with high-energy consumption,

accounting for at least 1% of global energy use. Around 80% of N_{\min} produced in the Haber-Bosch process is applied to soils as fertilizer (Erisman et al., 2008) but, N_{\min} use efficiency in agriculture is only around 33% (Raun & Johnson, 1999), with N use efficiency declining from ~60% to ~30% in the decades from the 1960s to 2000 (Erisman et al., 2008), indicating very high losses from soil. These N losses can have severe impacts on natural resources, as evident from losses of biodiversity, nitrate contamination of freshwater systems, emission of greenhouse gases to the atmosphere as well as soil acidification (Millennium Ecosystem Assessment, 2005). Furthermore, long-term N fertilization has been associated with changes to C cycling in soils (Neff et al., 2002) as well as losses of mineral-associated and subsoil organic carbon (Shahbaz et al., 2017). Supplementing or replacing agricultural N_{\min} with alternative N sources, such as naturally converted atmospheric N or recycled waste N, may therefore help to minimize damage to the environment.

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A prime candidate for alternative N sources are legumes, which have been used to enhance and maintain agricultural productivity since ancient times as they naturally fix atmospheric N into organic biomass. A portion of this N is then released into the soil during turnover of soil organic matter (SOM). The ability of legumes to substitute for N fertilizers and therefore reduce associated energy consumption was illustrated by Peoples and Craswell (1992), who found that green manure legumes generally provide N equivalent to 50–120 kg ha⁻¹, but individual systems may receive over 500 kg ha⁻¹ from just a few months of legume growth, with calculated N_{min} offsets equivalent to nearly 10,500 MJ ha⁻¹ energy consumption (Liebman et al., 2012). More recently, the use of legumes in crop rotation has received increasing attention due to their at times deep rooting systems, that promote biopore formation and thus provide access to subsoil water and nutrient resources (Gaiser et al., 2012; Kautz et al., 2013).

Including legumes in rotation has also been found to reduce C and N losses from agroecosystems (Drinkwater et al., 1998), as well as enhance SOM storage (Gregorich et al., 2001; Robertson et al., 2015), as biomass turnover leads to the incorporation of OC into soil. Whilst legumes therefore have potential to boost soil fertility, results are not consistent and legumes do not always enhance C storage (Robertson et al., 2015). This inconsistency in SOC enhancement is likely due to other limiting factors to plant growth, such as climatic conditions or stoichiometric constraints on biomass production (nutrient limitation). P fertilization, in particular, has been positively associated with legume nodulation and yields (Wani et al., 1995). Enhanced K availability has also been shown to stimulate red clover growth (Oram et al., 2014). The latter study showed that growth stimulation was only achieved under N limitation, highlighting the complex interactions in nutrient availability which affect legume growth. These studies highlight the need for more investigations aimed at disentangling the complex interactions between leguminous crops, nutrient dynamics and SOC storage, in particular for subsoils (Kautz et al., 2013), which are generally defined as lying below the A-horizon, i.e. the plough layer in agricultural soils.

One issue with identifying changes in subsoil OC storage is that subsoil OC contents tend to be low, whereas subsoil heterogeneity is high (Hobley and Wilson, 2016). This combination of low subsoil OC content, which is at times near the analytical limit of determination, and high variance results in a need to analyse more samples to detect the same changes due to management effects as in topsoil OC storage. To overcome these difficulties detecting differences in OC storage in subsoils, isotope analyses can be used to complement traditional chemical analysis (Hobley et al., 2017; Robinson, 2001). Such analyses are more sensitive than bulk OC contents, thereby improving analytical power.

In this study we evaluated the interactive effects of leguminous (crimson clover as green mulch, fava beans) and non-leguminous (maize) crops in rotation in combination with various inorganic fertilization regimes (no fertilization, PK fertilization, NPK fertilization) to assess the potential for additional SOC and N storage down the profile to 1 m depth. We hypothesized that legumes increase SOC and N storage, with benefits amplified by additional P and K fertilization, but reduced by additional N fertilization. However, the benefits of legumes on soil properties will be depth differentiated between crops, as clover green mulch is ploughed into the surface soil, whereas fava beans are harvested, so that N is primarily added via roots.

2. Methods

2.1. Site and soils

Sampling was performed in April 2016 at the Biological Nitrogen Fixation Experiment, located at and managed by the Justus von Liebig University, Gießen, Germany (longitude: 50° 36' 12' 'N, latitude: 8° 39' 16' 'E). The site is at 158 m above sea level and experiences a temperate climate (Köppen-Geiger climate classification Cfb) with a mean annual

Table 1

Fully factorial design of experiment with varied first year crop crossed with fertilization regime.

First year crop	Fertilisation regime		
Crimson clover	None	PK	NPK
Fava beans	PK	NPK	None
Maize	NPK	None	PK

temperature of 9.6 °C and a mean annual precipitation of 666 mm (long-term average from 1939 to 2017, Deutscher Wetterdienst, 2017).

The soil developed upon heavy textured, fluvial material and was classified as a eutric fluvic gleyic Cambisol (WRB 2015). The texture throughout the profile was determined using Micromeritics Sedigraph III Plus particle-size analyser on 28 samples chosen randomly over all treatments and depth intervals (see below). The texture was classified as silty clay, without a gradient from surface to subsoil (sand: 8 ± 4% mass, silt: 44 ± 3% mass, clay: 48 ± 5% mass). Rock content was negligible (median < 1% mass). The dominant mineralogical components were determined by X-ray diffraction using a Phillips PW1070 diffractometer, which identified quartz, numerous phyllosilicates and clay mineral components including illite and muscovite, chlorite, kaolinite and feldspar in all depths, with the relative abundance of the latter increasing with depth.

The experiment was established in 1982 as a fully factorial split plot design, with spatially randomized field replicates in four blocks. The first factor was a four-year crop rotation system, in which the first year crop was varied and included one of two legumes (crimson clover, *Trifolium incarnatum*, or fava beans, *Vicia faba*) or maize (*Zea mais*) (Table 1). The clover was used as a green mulch, with the entire biomass ploughed into the soil (plough depth ~30 cm), whereas the fava and maize were harvested, with the standing residues incorporated into the soil after harvest. The crops in years 2–4, were winter wheat (*Triticum aestivum* ssp. *aestivum*), winter rye (*Secale cereale*), and summer barley (*Hordeum vulgare*) respectively. The second factor was fertilization. Three fertilization regimes were employed, including a zero fertilization control ('no fertilization' or 'none'), a P and K fertilization without additional N ('PK'), and a full mineral fertilization comprising N, P and K ('NPK'). The fertilized plots received 90 kg ha⁻¹ yr⁻¹ P as triple superphosphate and 120 kg ha⁻¹ yr⁻¹ K as KCl. N fertilization was optimized to the winter wheat crop, receiving a total of 180 kg ha⁻¹ yr⁻¹ as calcium ammonium nitrate (to prevent acidification) in 3 doses. In year 1 (i.e. varied crop), no N fertilization occurred.

Each plot was 42 m² and each of the nine treatments (Table 1) was replicated in four blocks, resulting in 36 plots. The plots were sampled during a winter wheat crop using a hydraulic steel corer with an inner diameter of 6 cm. Two cores were taken from each plot to a depth of one metre and cut into four depth increments: 0–30 cm, 30–50 cm, 50–70 cm, 70–100 cm, yielding a total of 144 samples. These depths corresponded to the sampling scheme of the German Soil Inventory for Agriculture (<https://www.thuenen.de/en/ak/projects/agricultural-soil-inventory-bze-lw/>). Each depth increment was weighed in the field for bulk density calculations.

2.2. Sample treatment

Around 15 g of field fresh sample was weighed and dried at 105 °C to determine gravimetric moisture content. The remaining sample was dried at room temperature to constant mass. Dry samples were disaggregated using a jaw crusher prior to sieving at 2 mm. A 2 g aliquot of fine sample (< 2 mm) was ground using a Teflon ball mill to pass a 150 µm mesh for C, N and isotopic analysis.

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