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Do carbon farming practices build bioavailable nitrogen pools?

Tuomas J. Mattila[1] | **Andrei I. Girz**[1] | **Mari Pihlatie**[2]

[1]Finnish Environment Insitute SYKE, Helsinki, Finland

[2]Department of Agricultural Sciences, Institute for Atmospheric and Earth System Research (INAR), Viikki Plant Science Centre (ViPS), University of Helsinki, Helsinki, Finland

Correspondence
Tuomas J. Mattila, Finnish Environment Insitute SYKE, Helsinki, Finland.
Email: tuomas.mattila@syke.fi

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Abstract

Agricultural soils contain large amounts of nitrogen (N), but only a small fraction is readily available to plants. Despite several methods developed to estimate the bioavailability of N, there is no consensus on which extraction methods to use, and which N pools are critically important. In this study, we measured six soil N pools from 20 farms, which were part of a multi-year soil carbon sequestration on-farm experiment (Carbon action, 2019–2023). The aim was to quantify the N pools and to evaluate if farming practices that aim to build soil carbon pools, also build bioavailable N pools. We also aimed to test if the smaller and rapidly changing N pools could serve as an indicator for the slower change in soil organic matter. The measured N pools decreased in size, when moving from total N (7700 ± 1500 kg/ha) to slowly cycling (Illinois Soil Nitrogen Test ISNT-N: 1063 ± 220 kg/ha, autoclave citrate-extracted ACE protein N: 633 ± 440 kg/ha), water-soluble organic N (50 ± 17 kg/ha), potentially mineralizable N (33 ± 13 kg/ha) and finally readily plant available inorganic pools (nitrate and ammonium, total: 14 ± 8 kg/ha). In total, the measured pools covered only 18%–44% of total N, indicating a large unidentified N pool, which is either tightly bound to soil mineral fraction and not easily extractable or is bound to undecomposed plant residues and not hydrolysed by the methods. Of the large N pools (ISNT-N, ACE protein and unidentified residual N), clay, carbon (C) and C:Clay ratios explained most of the variability (R^2 = .90–.93), leaving a minor part of the variation to the management effect. A pairwise comparison of carbon farming and control plots concluded that farming practices had a small (3%–5%) but statistically significant (p < .05) effect on soil total N and ISNT-N pools, and a moderate and significant effect (18%, p < .01) on potentially mineralizable N. The large variation in protein N, water-soluble organic N and inorganic N reduced statistical significance, although individual C sequestration practices had large effects (−30% to +50%). In conclusion, carbon sequestration practices can build both slowly cycling N pools (ISNT) and increase the mineralisation rate of these pools to release plant available forms, resulting in an additional benefit to agriculture through reduced fertilizer application needs.

KEYWORDS

ACE protein, carbon farming, glomalin related soil glycoprotein, GRSP, ISNT

1 | INTRODUCTION

Agricultural soil contains large amounts of nitrogen (N), but only a small fraction is readily available for plants. Different methods have been developed to analyse the release of plant available N from larger pools (Hurisso et al., 2018), but there is no consensus on which extraction method to apply or how to evaluate the availability of different pools (Geisseler et al., 2019). Pools such as nitrate (NO_3^-) and ammonium (NH_4^+) are readily available, and the water-soluble organic N (WSON) pool can decompose to supply N to plants (Murphy et al., 2000). However, the size of these soluble pools is small compared with plant uptake amounts. They are also unstable, changing constantly through mineralisation, uptake and immobilization. More stable N pools, bound to organic matter or mineral soil fraction, can be used for tracking soil N supply potential, but the lack of standardized methods limits their interpretation. For example, the autoclave citrate-extracted protein (ACE protein) pool was thought to represent glomalin, a thermostable and recalcitrant glycoprotein present in large amounts in some soils (Holátko et al., 2021). Later, in addition to glomalin, the ACE method was demonstrated to extract various other compounds as well and that the pool sizes have been overestimated (Moragues-Saitua et al., 2018). The "glomalin related soil proteins" (GRSP), even though unknown in composition, can still be a useful measure of bioavailable organic N (Hurisso et al., 2018). Similarly, the Illinois Soil Nitrogen Test (ISNT) alkali hydrolyses a combination of organic N compounds ranging from bacterial aminosugars to amides (Kwon et al., 2009), and can serve as a good predictor of N supply to plants (Braos et al., 2022; Khan et al., 2001). These large and slowly bioavailable N pools respond to management (Klapwyk et al., 2006; Liu et al., 2020), making them a good indicator for tracking soil change.

The interpretation of the different N pools becomes easier when they are connected in a conceptual framework. Daly et al. (2021) presented an organic N framework, which emphasizes the role of particulate and mineral associated organic matter (POM and MAOM) as sources of bioavailable organic N (Figure 1). Organic N is supplied from POM by depolymerization and solubilization and from MAOM through sorption–desorption. When the supply from POM exceeds the sorption potential to MAOM, more loosely bound and bioavailable organic N compounds accumulate, supplying N for plants and microbes. The framework links the N cycle to emerging topics in carbon cycling and can also explain the strong effect

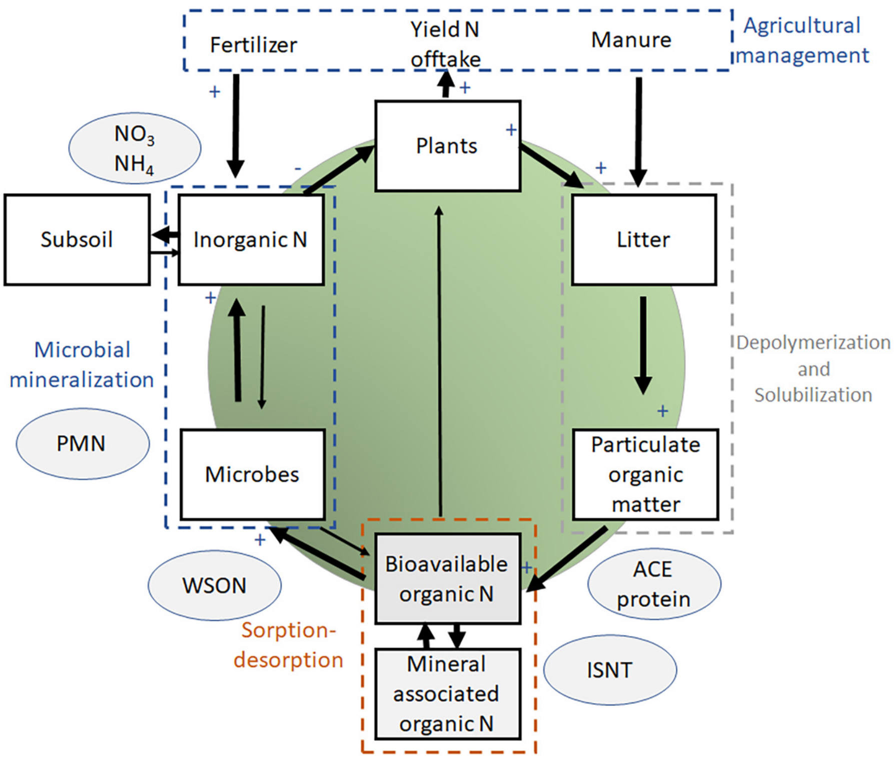

FIGURE 1 Agricultural management controls the nitrogen cycle by managing plants and by supplying extra N to the soil. Increased plant biomass will also result in an additional build-up of bioavailable organic N and stimulate mineralisation of inorganic N. The boxes represent pools and the arrows flows between the pools. The size of the arrow indicates the magnitude of the flow. The grey circles represent N pools measured in this study (ACE, autoclave citrate-extracted protein; ISNT, Illinois Soil N Test; NO_3, NH_4, inorganic N; PMN, potentially mineralizable N; WSON, water-soluble organic N). Modified from the framework of Daly et al. (2021).

that OM: clay ratios can have on carbon and N cycling (Prout et al., 2022; Soinne et al., 2021). It also emphasizes the earlier conclusion, that instead of mineralisation, depolymerization is the rate-limiting step for supplying N to plants (Jan et al., 2009).

Various N pools have been successfully used to predict N supply to plants. A potentially mineralisable N (PMN) estimation based on WSON and a 24-h aerobic incubation ("CO_2 burst") can predict N mineralisation over multi-month soil incubations (Franzluebbers, 2020). Predicting N mineralized over the growing season has made it a popular tool to make more accurate fertilizer recommendations (Haney et al., 2018; Harmel & Haney, 2013). The "CO_2burst" method is based on readily bioavailable N pools (WSON, microbially available N and inorganic N), but nitrogen supply to crops can also be estimated from the more slowly cycling N pools (ISNT and ACE protein) (Braos et al., 2022; Geisseler et al., 2019). The ISNT-N test can differentiate if crops would respond to N fertilizer or not based on a proposed threshold of 230 mg/kg of ISNT-N (Khan et al., 2001). However, calibration is needed to interpret the results in different soils and climates (Braos et al., 2022). When N supply from organic sources has been included in fertilizer planning, the results have been promising: yields have been maintained with 30%–50% reduction in fertilizer N inputs (Haney et al., 2018). As the N balances of USA, China and EU have been 30–50 kg/ha/year positive throughout the 2000s (OECD, 2020; Zhang et al., 2015), a soil based approach to N fertilization offers great promise for reducing excess N fertilization and mobilizing the accumulated soil N.

Reducing the N balance is not the only soil management challenge for the 21st century. Soil carbon sequestration has been identified as a key climate mitigation tool (Chenu et al., 2019). Building soil carbon pools through carbon farming practices such as cover crops, reduced tillage, crop rotations and soil amendments can remove considerable amounts of atmospheric CO_2 to soil C (Chenu et al., 2019). Simultaneously, concerns have been raised that the build-up of C in soil organic matter (SOM) also requires a build-up in N (Poulton et al., 2018; Rumpel et al., 2019), possibly requiring additional fertilization. However, deep-rooted crops, legumes and cover crops used in carbon sequestration (Paustian et al., 2019) can bring the potentially leaching inorganic N into the organic N cycle (Aronsson et al., 2016; van der Pol et al., 2022). In theory, soil C sequestration would also result in the build-up of soil N reserves, but the bioavailability of those new reserves is unknown.

In this study, we analysed 40 soils from an on-farm carbon sequestration experiment to see if 4 years of "carbon farming" had increased soil labile N pools. The farms had applied carbon sequestration practices such as cover crops, clover leys in rotation, diverse grass crops, subsoiling, soil amendments and adaptive multi-paddock grazing. The farms spanned the major agricultural areas of Finland and a wide range of mineral soils. The aim was to (i) quantify the bioavailable N pools, (ii) evaluate the effect of carbon farming on different N pools, and (iii) to quantify the potential fertilizer reductions from increased supply of N from soil to plants.

2 | MATERIALS AND METHODS

2.1 | Fields and sampling

The sampled fields consisted of 20 farms selected for intensive annual monitoring from a group of 105 farms in the Carbon Action experiment (Mattila et al., 2022). The farms tested six different carbon farming practices (cover crops, leyfarming, improved grazing, soil amendments, subsoiling and diverse grass mixtures) on their fields. The intensive monitoring farms were chosen to represent all the carbon farming practices, on different soil types and farming systems (cereal farming, mixed livestock and vegetables). An additional requirement for the monitoring farms was that they should also have a good evaluation report on the experimental design by an independent expert panel, as described in Mattila et al. (2022). The selected farms were located in a 200 × 500 km area which covered the main agricultural areas of Finland (ranging from 63.18 N to 60.36 N in latitude). The aim was to have four replicates for each carbon farming measure (cover crops, leyfarming, improved grazing, soil amendments and subsoiling), but we got only three to the grazing study within the study area and two of the subsoiling farms moved to diverse grass group as weather conditions prevented subsoiling. Each study farm had an experimental field, which was split into two experimental units: control and carbon farming. The experiment was farmer-led: the farmers were guided to design a management plan for the carbon farming (CF) field and maintained current farming practices (CP) on the control side. The aim of research was to document the changes caused by the farming practices over time. This was a methodological difference compared with a more strict, controlled experiment, but represents practices and challenges in actual agricultural operations. The soil types were mainly silty clay loam, with clay contents of median 37% (6%–60% confidence interval) and organic matter of median 6.4% (2.6%–10.3%) (Figure 2). Regarding farming systems, 30% of the farms were certified organic and 25% of the fields were in grass farming during the experiment.

The farmers kept notes of the management actions done in the fields. For this study, we used the data from

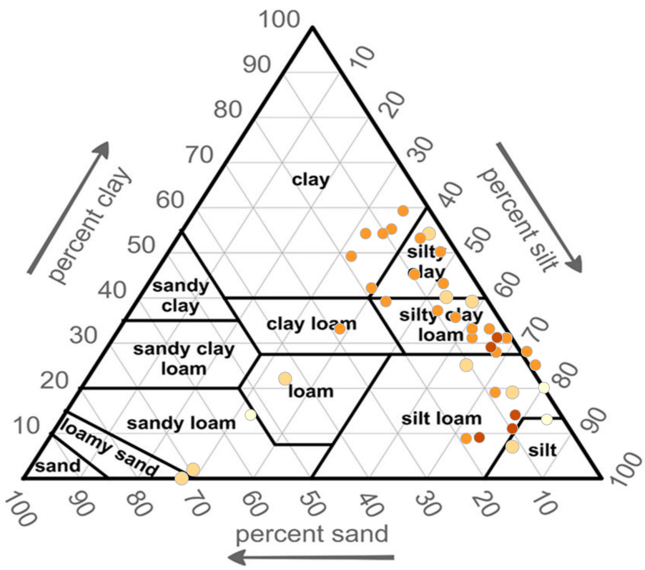

FIGURE 2 Soil types in the experimental fields. The darker colours indicate a higher organic matter, ranging from 2.2% to 17.7%.

fertilizer applications in 2022 spring, manure and amendment applications in autumn 2021, and the yield harvested in fall 2022. This was used to construct a nitrogen balance, the details of which are given in Section 2.3.

The fields were sampled in July 2022, 4 years after the start of the experiment in 2019. Each field had 3 static GPS points, which were the centre point for sampling. Ten cores were collected at a 10 m radius from the static GPS point using a 17 mm soil corer with depth markings for 0–17 cm (topsoil) and 17–30 cm (subsoil). This division represented historical tillage depths of 16–20 cm. The cores from each point ($n = 3 \times 10$ for subsoil and topsoil) were pooled for each experimental unit so two separate pooled composite samples (topsoil, subsoil) represented each field. In addition, we collected biomass samples from each field using a 31 × 31 cm sampling square: six sampling quadrats were collected from each field, the fresh sample was weighed, and a subsample was taken for dry matter determination (oven drying). The biomass samples were used to evaluate N uptake to the plant matter and the ratio of N taken up versus what left the field with harvested yield. Subsoil and biomass samples were not collected from two farms because of a thunderstorm during sampling, resulting in 76 soil samples and 36 biomass samples for the dataset.

2.2 | Analysis methods for soil nitrogen pools

We used high throughput and highly scalable methods for quantifying the N pools. As the farms were a subset of a larger 105 set of farms, maintaining the potential to scale the experiment later and to also provide a practical tool for farmers and advisors was a priority. Therefore, we used ISNT-N instead of the more detailed methods of fractional hydrolysis (Kwon et al., 2009) for example. The pools were defined operationally, for example, the ISNT-N represented the N compounds which were converted to ammonia in a short-term acid incubation, and the ACE protein represented N compounds which survive high temperatures and still react with a Bradford protein assay. This operational classification of pools resulted in some overlap between pools, for example, the potentially mineralizable N is derived from WSON, which is partially represented by ISNT-N. For the largest two pools (ISNT-N and ACE protein) the overlap was minor, as ISNT-N mainly detects bacterial aminosugars and amides (Kwon et al., 2009) and the Bradford assay in the ACE protein analysis does not react with small molecular weight proteins or amino acids. We aimed to analyse distinct bioavailable pools, but we could not avoid some overlap.

Measurement of water-soluble inorganic and organic N could be done with routine methods. For the inorganic N, we used fresh soil samples, refrigerated for 2 days at 2°C (to maintain the same cold incubation time for all samples, which were sampled from Monday to Wednesday every week). After refrigeration soils were extracted 1:5 with 2 M KCl, filtered to 2.5 μm (Whatman 42), and frozen for later analysis. The NO_3-N and NH_4-N contents were analysed colorimetrically with a discrete analyser (Thermo scientific gallery Plus). Water-soluble C and N (WSOC and WSON) were analysed by water extraction of the dried and sieved sample (<2 mm). Soil was extracted 1:10 with milliQ-water, filtered to 2 μm and stabilized with HCl stabilization. The analysis was conducted as for routine water samples (Lachat FIA QC 8500/SFS-EN ISO 11905-1:1998; Shimadzu TOC-V_{CPH}; standard SFS-EN 1484:1997). Inorganic N was subtracted from the water-soluble total N to provide water-soluble organic N. The total C and N were also measured from a dried and sieved soil sample with an elemental analyser (dry combustion Leco CN-2000, 0.15 g sample size).

Analysing ISNT-N required building an apparatus for soil alkali hydrolysis and diffusion. In the test N is released from organic matter in a 5-h alkali hydrolysis (2 M NaOH), volatilized to ammonia and captured by boric acid (Khan et al., 2001). The ammonia is then titrated from the boric acid with sulphuric acid and the N-release amount is calculated from the titrant consumption, as in the familiar Kjeldahl procedure for total N (Martín et al., 2017). For the ISNT-N, we followed the procedure of (Spargo & Alley, 2008), who modified the original hot-plate method to use an incubator instead and lengthened the incubation time (15 h). These modifications result in nearly identical results compared with the original hot-plate method (less than 1% difference), but the modified method is simpler to operate, has higher throughput and is more precise (Spargo & Alley, 2008). Following Spargo and Alley (2008), we fitted a Petri dish with 5 mL of 4% boric acid to the lid of a 700 mL jar, applied 1 g of soil and 10 mL of 2 M NaOH into the jar and incubated for 15 h at 50°C. The boric acid was titrated with 0.01 M H_2SO_4 to reach pH 4.3. We replicated 16 of the 76 measurements to determine measurement error, which was on average 6 mg N/kg soil or 0.7% of the measured result. To avoid double counting inorganic N, we subtracted water-soluble inorganic N readings from the ISNT-N result.

The autoclave citrate-extracted protein (ACE protein) was analysed with readily available equipment (microplates, autoclave, centrifuge, Bradford protein assay) but with a highly customized protocol (Hurisso et al., 2018). To avoid the problems from co-extraction of humic substances, we applied a colour correction as in (Cissé et al., 2020; Moragues-Saitua et al., 2018). A subsample of 1.25 g of dry soil was sieved to 0.5 mm, and 10 mL (1:8 soil: extract) sodium citrate (20 mM) was added and the mixture was adjusted to pH 7 with HCl. The soil was autoclaved at 121°C for 30 min, centrifuged (15,000 g, 10 min) and the supernatant was centrifuged again to settle clay particles. This corresponded to the "easily extractable glomalin related soil protein" pool (Wright & Upadhyaya, 1996), which consists of proteins that are extracted by the citrate and not denatured by the autoclave. To avoid overestimation of the protein amount by the spectrophotometric method in the Bradford assay, the colour correction by (Cissé et al., 2020) was used. A sample blank was used, which included the extracted sample, but not the Bradford reagent and was adjusted to the same pH as the Bradford reagent added sample. To avoid double counting the well, a buffer-filled well was included and added to the measurement (i.e. total measurement $A_{\text{protein}} = A_{\text{bradford}} - A_{\text{coloured+well}} + A_{\text{well}}$) (Cissé et al., 2020). If the absorbance was too high compared with the standards (bovine serum albumin, BSA 0–350 mg/L), the samples were diluted (1:2 or 1:3) to maintain the analysis on the linear standard curve. The analysis was done in a 96-well microplate, using 10 μL of the sample, 10 μL of sodium citrate buffer, and 230 μL of Coomassie Protein Assay reagent (Bradford, Thermo scientific) or 230 μL of 0.1 M HCl for the sample blanks. After the addition of the reagent, the plate was covered with film, tapped against the table for 1 min, centrifuged at 660 g for 2 min and read at 595 nm (Tecan Spark plate reader). The error between the replicated measurements was 3% or 7 mg N/kg.

The measurement of potentially mineralisable N (PMN) required the use of a customized commercial respirometer (Solvita IRTH respirometer). We used 24-h aerobic incubation ("CO_2 burst") to determine potentially mineralisable N (PMN) (Franzluebbers et al., 1996; Haney et al., 2018). The CO_2-C respired following drying/rewetting was divided by the water-soluble C to estimate bioavailability (% microbially active carbon) and then the water-soluble N was multiplied with this bioavailability to estimate N release from a single respiration pulse. Multiplying with the number of rewetting pulses in a growing season (set to 5.5 to match with earlier PMN estimates; Mattila & Rajala, 2020) gave an overall PMN estimate in mg N/kg soil (Haney et al., 2018). As the process is sensitive to sample processing, we used the latest recommendations for rewetting and sample treatment (sieving to <2 mm, no grinding, and wetting to 50% pore volume) (Franzluebbers & Haney, 2018).

All the methods produced N concentrations per soil mass (mg/kg soil). These were converted to soil N amounts (kg/ha) by multiplying with representative volume (sampling depth [m] times area [m^2/ha]) and bulk density [kg/m^3]. We only had bulk density samples collected from 0 to 5 cm, so we used a previously published, locally calibrated pedotransfer function ($\rho = 1.52 - 0.280 \times \ln$ (C-%)) to estimate soil bulk density from the carbon content (Heikkinen et al., 2020). The bulk density was estimated separately for the two sample layers (0–17 cm, 17–30 cm) and the layers were combined for evaluating N stocks in the 0–30 cm soil profile.

2.3 | Data analysis and statistical testing

The measured soil N pools were used as they were, but the collected biomass and farmer management actions needed processing before analysis. The biomass was converted to N stock by multiplying with typical N values from the species and growth stage (Bryson & Mills, 2015). The N balance was calculated using $NB = N_{\text{fert}} + N_{\text{manure}} \cdot x - \text{Yield} \cdot f_N$, where N_{fert} was the applied mineral fertilizer, N_{manure} was the N in manure, x was an availability factor (Sullivan et al., 2010) based on the N content of manure, Yield is the harvested yield, and f_N was the fraction of N in the harvested yield. The fraction of N in the harvested yield was obtained from an IPNI database (IPNI, 2014). For the plant species that were not in the IPNI database, protein contents from a feed database (LUKE, 2014) were converted to N by assuming 16% of N in protein. The yield was straightforward for cereals and harvested silage, but for the grazing plots, we assumed that a grazing animal removes 3% of body weight as dry matter feed intake (Coughenour, 2012). We also assumed that 40% of the feed intake was deposited as manure (Luostarinen et al., 2018). In addition to the N balance, we calculated a "nitrogen harvest index" $N_{\text{yield}}/N_{\text{biomass}}$ to evaluate how large a fraction of the N taken up by the plant in the aboveground biomass was later translocated to the harvested yield.

We used three kinds of statistical tests for the analysis: Pearson's r, multiple least squares regression and Wilcoxon signed-rank test. Pearson's r was used to investigate the correlation between different N pools and between N pools and soil properties. To evaluate the role of soil properties versus management, we constructed linear models of clay, carbon and clay: carbon-ratio to explain the variation in the observed N concentrations. The clay:-carbon ratio is an important factor for both soil structure and carbon change (Prout et al., 2022). High clay:carbon ratios (>18) can also suppress N mineralisation (Soinne et al., 2021). Linear models of clay, carbon and carbon: clay ratio were fitted to data with least squares regression and the model fit was evaluated through the coefficient of variation (R^2), residual error and Cook's distance for outliers Third, to evaluate the effect of carbon sequestration practices, we calculated the response-ratios between carbon farming and current practice ($RR = X_{CF}/X_{CP} - 1$, where X_i is the investigated parameter) and tested if the RR was statistically significantly different from zero with a Wilcoxon signed rank test. The analysis could normalize the large variation in the pool sizes between farms but did not correct for the effect of total carbon or clay, which could vary between treatment and control plots.

All the statistical analyses were conducted in R statistical software, and the graphics were drawn with the *ggplot2* library.

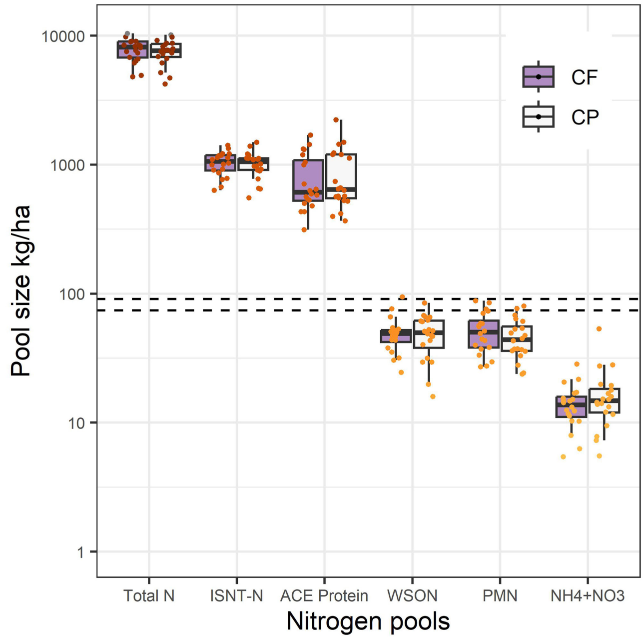

FIGURE 3 Soil nitrogen pools in the 40 fields separated by carbon farming (CF) and current practise (CP). The dotted lines are the median plant N uptake (91 kg N/ha) and crop removal (74 kg N/ha) (ACE protein, autoclave citrate-extracted protein; ISNT-N, Illinois Soil Test N; NH_4+NO_3, inorganic N; PMN, potentially mineralizable N; WSON, water-soluble organic N).

3 | RESULTS AND DISCUSSION

3.1 | Three organic N pools: large, medium and small

The soils under study had considerable pools of nitrogen (Figure 3), which could be classified by their relative size. The total N pool was the largest (7700 ± 1500 kg/ha), followed by two medium-sized pools ISNT-N (1063 ± 220 kg/ha) and ACE Protein N (633 ± 440 kg/ha), and two smaller pools: water-soluble organic N (50 ± 17 kg/ha) and potentially mineralizable N (50 ± 18 kg/ha). The amount of inorganic N (NO_3+NH_4) was small but highly variable (14 ± 8 kg/ha). The topsoil sample was slightly larger than the subsoil (17 vs. 13 cm), so it was 57% of the total sample volume. The larger pools ISNT, Protein N and Total N had a similar distribution to sample volume (62 ± 2% of the total amount in topsoil), indicating an even distribution and lack of stratification. The smaller pools of WSON and PMN were accumulated in the topsoil (66 ± 7% and 72 ± 8%, respectively). The readily plant-available Inorganic N was found evenly in top- and subsoil, but with high variability, which reflected fertilization and plant uptake (63 ± 12% in topsoil). When compared with plant N uptake (ca. 100 kg N/ha), the pools are considerable and if they would be made plant available, they could replace a large fraction of soil fertilizer needs (Haney et al., 2018).

The ISNT-N was the largest of the specified N pools. It is thought to represent soil bacterial aminosugars (Kwon et al., 2009) and has been linked to crop responsiveness to N fertilization (Khan et al., 2001). Compared with the threshold for non-fertilizer-responsive soils (230 mg/kg) (Khan et al., 2001), 62% of the study soils were higher than that. However, the threshold was determined for corn in Illinois (40 N latitude) while our study soils were shorter season crops in Finland (60 N). The cooler climate and shorter growing season limit the N supply from the ISNT-N pool. Nevertheless, the measured ISNT-N concentrations were very high (106–506 mg/kg our study; 72–435 mg/kg in Khan et al., 2001; 102–371 mg/kg in Spargo & Alley, 2008; 250–350 mg/kg in Klapwyk et al., 2006). The higher ISNT-N concentrations were found on soils with high clay (>37%), carbon (>5%) or recently applied manure. On average the ISNT-N was 13.3 ± 0.7% of total N. This was much lower than in previous studies 11%–22% (Spargo & Alley, 2008) or 11%–27% (Khan et al., 2001). However, our samples were much higher in organic matter than those studied earlier (max. 2.7% in Khan et al., 2001, 2.5% in Spargo & Alley, 2008, and max. 7.4% in our study, with 60% of samples exceeding 2.5% C). The low fraction of total N extracted by the ISNT could indicate that there is a large pool of organic N in the studied soils, which is not hydrolysed by the alkali treatment in the ISNT test.

Soil autoclave citrate extractable (ACE) protein is an operationally defined pool of soil N, which is also known as "glomalin-related soil protein" (GRSP) (Cissé et al., 2020; Hurisso et al., 2018). It was initially thought to be a stable "glomalin" like compound but is increasingly being identified as a constantly cycling N pool (Cissé et al., 2021), which can be used to predict N supply to crops (Geisseler et al., 2019). In our study soils, the pool of ACE protein N was highly variable (169 ± 165 mg N/kg) and represented 11 ± 6% of total N (maximum 29%). This was slightly lower than in Geisseler et al. (2019) (28% on average and 67% maximum; Geisseler et al., 2019) but higher than in Halvorson and Gonzalez (2006) 6.5%. The highest share of ACE in our study was in soils with very low clay content (4%–6% clay). The concentration of ACE was lower than in previous studies (Fine et al., 2017 or Geisseler et al., 2019). This could in part be explained by the high percentage of clay in our study soils, as ACE levels are twice as high in coarse soils compared with clay loams (Fine et al., 2017). We also used the recent colour correction (Cissé et al., 2020, described in methods Section 2.2), which reduced the inference from coloured humic substances, being highlighted as a challenge by Geisseler et al. (2019) and Halvorson and Gonzalez (2006). This corrected the problem found by Alex McClellan et al. (2022), where Bradford analysis of soil protein resulted in over 400% of total N detected as protein. The concentrations we measured were similar to those found in the natural revegetation of farmland in the Loess Plateau (Liu et al., 2020), which were measured using the same colour correction. Overall, using the colour-corrected value, the soil ACE represented a similar magnitude pool to ISNT, but the two pools were only weakly correlated and differed in their relation to texture. The ISNT increased with clay content and the ACE protein decreased (Table 1).

The two smaller organic N pools (water-soluble organic nitrogen WSON and potentially mineralizable nitrogen PMN) are thought to represent readily bioavailable N pools. The WSON is a product of depolymerisation of plant residue and soil proteins and the release of mineral-associated N to a bioavailable form (Figure 1). The WSON is then readily available for microbial decomposition, feeding to the PMN pool. In calculation of PMN, the fraction of WSON mineralized was estimated from the fraction of WSOC respired as CO_2 during the burst experiments. This fraction was 15.5 ± 6%, indicating a low bioavailability of WSOC, perhaps caused by the very high supply of WSOC from the large total C pool. Compared with total N, the PMN was 0.5 ± 0.2%, which is very similar to the C decomposition rates (0.6% of C pool per year) estimated for a similar climate (Andrén & Kätterer, 1997). This highlights the scale of PMN and the relatively slow cycling of most of the total N pool. At the same time, the PMN was three times larger than inorganic N pools, confirming the importance of organic N in supplying N to plants.

The WSON pool was only 50 ± 16 kg N/ha (13 ± 6 mg/kg), approximately twice the size reported for agricultural soils of England (Murphy et al., 2000). Although the WSON was three times larger than the amount of inorganic N, it was still much smaller than plant N uptake (91 kg N/ha). However, this relatively small pool (thought to consist mainly of amino acids and their oligomers; Warren, 2014) is rapidly being replenished by the larger pools and is being mineralized to form NH_4 and NO_3 or immobilized into mineral

TABLE 1 Correlation (Pearson *r*) of the nitrogen pools with each other, with soil properties and carbon pools.

	Total N	ISNT	Protein	WSON	PMN	MinN
Total N	**1.00**					
ISNT	**0.98*** **	1.00				
Protein	0.43***	0.46***	1.00			
WSON	**0.72*** **	**0.71*** **	0.41***	1.00		
PMN	**0.59*** **	**0.60*** **	0.32**	**0.73*** **	1.00	
MinN	0.05	0.02	0.22	−0.05	0.08	1.00
Total C (%)	0.29**	0.34***	**0.61*** **	0.14	−0.06	−0.23
Clay (%)	0.28**	0.24*	**−055*** **	0.09	−0.01	−0.23
WSOC	**0.81*** **	**0.80*** **	0.28*	**0.80*** **	0.43***	−0.09
C/N	0.25*	0.30**	**0.86*** **	0.25*	0.11	0.06
WSOC/WSON	−0.25*	−0.25*	−0.29*	**−0.65*** **	**−0.64*** **	−0.08
C:Clay	−0.06	−0.03	0.71***	−0.09	−0.08	0.14

Note: Strong correlation (>0.5) presented in bold.

*0.05

**0.01

***0.001 significance level.

surfaces (Figure 1) (Murphy et al., 2000). In our samples, the WSON did not correlate with total C but correlated strongly with total N and ISNT. The WSON pool was 0.6 ± 0.2% of total N and 4.8 ± 1.5% of ISNT-N. The highest WSON:ISNT ratio (6%–9%) was on soils, which have had manure applied in the spring of 2022 before sampling in July. Some have suggested that soil N supply to plants is limited by the hydrolysis of proteins to amino acids and not by the mineralisation of bioavailable amino acids to inorganic N (Jan et al., 2009; Noll et al., 2019). The strong correlation between ISNT and WSON and between WSON and PMN would support this idea of labile N being converted to bioavailable forms, which then drive mineralisation. However, the weak correlation between ACE protein N and WSON and PMN would indicate, that ACE protein does not represent the N pool that is being solubilized to WSON and that ISNT would be a more reliable measure of the moderately labile N pool.

There was no correlation between inorganic N and any of the measured organic N pools (Table 1). Inorganic N was not influenced by the soil properties or carbon pools. Overall, as the soil inorganic N pool has a rapid turnover in agricultural soils (Butterbach-Bahl et al., 2013) because of plant and microbial uptake or releases, the pool size poorly represents the actual turnover. Over 25 kg/ha inorganic N was recovered only from sites where the crop had failed because of drought or where the fertilization of a late-seeded brassica crop had happened a few weeks earlier. For the sites with plant cover, regardless of fertilization or manure application, the amount of inorganic N in the soil was very low. This indicated that fertilizer applied in May had mostly been taken up by July and that plant N uptake controlled the concentrations. Most of the inorganic N was recovered from the topsoil (average 9 ± 7 kg/ha vs. 4.5 ± 2 kg/ha in the subsoil). The sampling was performed in July, during maximal crop N uptake in Finland. The results highlight the importance of soil organic N pools in supplying the crop with N, which is discussed further in Section 3.3.

A puzzling aspect of the results was that the total amount of identified N pools covered only 26 ± 6% of the total N. The non-identified N pool correlated with clay and total C, but it is unclear, whether it represents small organic molecules tightly bound to mineral matter (MAOM) or partially undecomposed plant residues (particulate OM, POM). In mineral soils, POM is usually only <20% of total N (Daly et al., 2021), so most of the unidentified N is likely to be MAOM.

3.2 | Are N pools controlled by texture, carbon or management?

The strong correlations of the N pools with inherent or slowly changing soil properties (carbon and clay) raised the question of their applicability to track management-induced changes. If clay and carbon explained most of the observed variability, a 4-year experiment had not changed the pools significantly. Indeed, in the Loess plateau revegetation study (Liu et al., 2020), there were some changes in soil ACE protein in the first 7 years, but the most rapid changes were found after 22–32 years. In contrast, the application of manure can change the ISNT in a matter of weeks (Klapwyk et al., 2006).

Taken together, clay, carbon and clay: carbon explained the variation in Total N (R^2 = .90), ISNT-N (R^2 = .90), ACE protein (R^2 = .93) and the non-recovered N pool (Total N – identified pools; R^2 = .83) very well, but failed to explain the variation in WSON (R^2 = .50), PMN (R^2 = .21) or inorganic N (R^2 = .20). The linear models for the three largest N pools and the sample data points are presented in Figure 4. For fitting those models, two outlier fields had to be excluded, one had a large amount of organic N (>250 kg total N/ha) applied in the previous autumn and the other had manure applied in the spring before sampling. These outliers were identified through Cook's distance in the model fitting and removed from the fitting dataset, but were used to evaluate the model fit. The model error was 10% of the median value for total N, 14% for the unidentified N, 11% for ISNT, 28% for ACE protein, 33% for WEON, 58% for PMN and 65% for inorganic N. The excellent fit for the more stable N pools and the very poor fit for the more labile N pools suggest that the more labile N pools are controlled by management, and that the more stable pools had not changed sufficiently to override the effect of soil properties. As clay and carbon and the sole and most important sinks for soil N, it was not surprising, that the amount of these sinks could control the formation of the more stable N forms, which are then protected from immediate decomposition by microbes. On the other hand, the "sink" terms could not explain the rapidly fluctuating bioavailable pools, which were then controlled more by agricultural management and plant growth.

An interesting observation on the shape of the linear functions (Figure 4) is that the trend for clay is inverted for protein and the other N pools. The higher the clay concentration, the lower the ACE protein concentration. This supports the earlier findings, where clay content controls the ACE protein amount across soils in the US (Fine et al., 2017). The shape of the function is also curved, indicating the importance of the C:Clay term in defining the function (if it would not be important, the contour lines would be straight lines). The C:Clay term was highly significant for all models in Figure 4, and the only term that was not significant was the impact of clay-% to ISNT (as it was already partially covered by the C:Clay term).

Based on the pairwise comparison, carbon farming had a statistically significant effect only on Total N (soil amendments), ISNT (cover crops), potentially mineralizable N

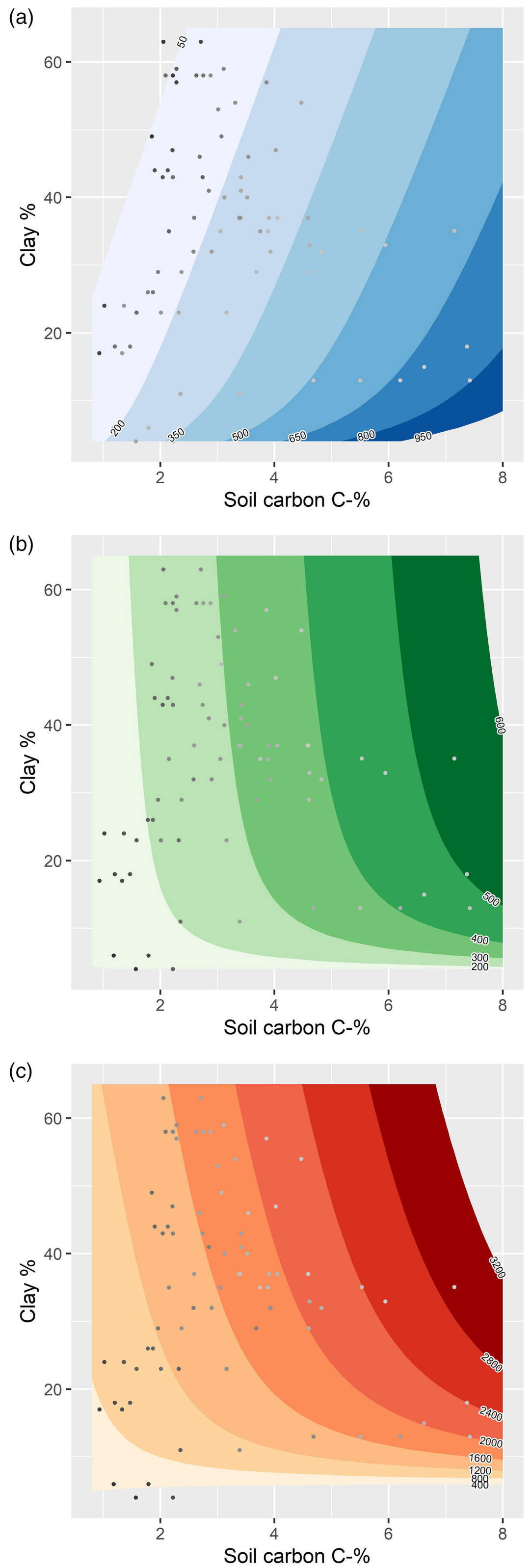

FIGURE 4 A contour plot of the multiple linear regression models: Clay content and soil total C explained most of the variation in (a) Protein-N (R^2 = .93), (b) ISNT-N (R^2 = .90) and (c) Unidentified N (R^2 = .83). The points represent measured soils (N = 76) and the darkness of the points represent the size of the pool. The darker background colours are increasing levels of the N pool predicted by the model.

(leyfarming), protein (subsoiling) and MinN (subsoiling) (Table 2). The variation in the response between farms was so large that it resulted in non-significant results for the groups that had a strong effect (>20%) on N pools (effect of leyfarming and soil amendments on labile N pools: WSON, PMN and MinN). This demonstrated that management can affect the levels of labile N pools, but that the direction is not guaranteed. The 4-year carbon farming duration was not enough to create a significant difference for the more stable N pools (Total N, ISNT and ACE Protein).

3.3 | Implications for agricultural management

Carbon sequestration practices had caused a notable difference in the plant N uptake. The silage and pasture plots contained nitrogen-fixing plants, making it impossible to estimate soil-supplied N from the N balance (on average 100 ± 96 kg N/ha in biomass vs. applied in fertilizer), but for the cereal crops the comparison was more straightforward. For the carbon farming plots, the N balance for cereal grains was negative (−21 ± 14 kg/ha) and the N in the biomass in July was 37 ± 19 kg/ha higher than what was applied in fertilizer. This indicated that the soil supplied a considerable amount of N to the crop (ca. 30% of N (10%–80%) in the harvested crop). The harvest index (N in biomass in July vs. N harvested) was 91 ± 5%, indicating that crop uptake of N had happened before July, and that the crop could translocate the N to the yield efficiently. For the control plots (current practice), the N balance was similar to the treatment plots (−24 ± 14 kg/ha) as was the N in the biomass in surplus to applied fertilizer 44 ± 26 kg/ha. The N harvest index was of similar magnitude, but much more variable 95 ± 28%, indicating that in some cases the crop continued N uptake after July and in others the crop was unable to translocate the N to the crop. At the same time, as much of the N in the above ground biomass was removed by the harvested yield, very little N was left for building soil N pools (ca. 6 kg N/ha and the N taken up from July sampling to August harvest). In contrast to this small annual input, the N input from leys in rotation can be considerable (the largest N input from a green manure clover ley was ~280 kg N/ha in this experiment). Even undersown cover crops can capture 30–50 kg N/ha and convert it into an organic form (Aronsson et al., 2016).

In both the control and treatment sides, the amount of N taken up by the plant from non-fertilizer sources was of similar magnitude to the PMN (33 ± 13 kg/ha). However,

TABLE 2 Response of soil nitrogen pools to management actions ("carbon farming practices"), (Response ratio %: (treatment/control − 1) × 100; the colours describe the direction and magnitude of change: red colour means lower and blue means higher N pool in carbon farming side).

N pool	Soil amendments	Cover crops	Diverse grass	Grazing	Leyfarming	Subsoiling	All
Total N	10 ± 8*	8 ± 9	6 ± 1	2 ± 5	6 ± 11	−3 ± 9	5 ± 9*
ISNT	5 ± 7	6 ± 8*	3 ± 2	3 ± 7	7 ± 10	−7 ± 7	3 ± 9*
ACE Protein	−7 ± 16	4 ± 16	0 ± 4	−4 ± 8	−7 ± 17	−11 ± 6*	−4 ± 14
WSON	20 ± 49	−6 ± 21	−2 ± 11	8 ± 20	45 ± 90	−3 ± 18	12 ± 48
PMN	32 ± 56	11 ± 23	4 ± 19	−7 ± 23	54 ± 60*	1 ± 10	19 ± 43*
MinN	−19 ± 31	11 ± 19	10 ± 13	36 ± 41	−34 ± 65	10 ± 5*	−1 ± 41

*0.05 significance level.

the correlation was very weak (R^2 = .07, p > .05), suggesting that other factors than N availability have limited the yield. As 2022 was an exceptionally dry and hot year in Finland, water limitation may have occurred. Therefore, the role of soil PMN in forecasting N supply to crops could not be evaluated in this experimental set-up, which contained both nitrogen-fixing crops and an exceptionally dry growing season. The overall scale of N uptake from non-fertilizer sources was of similar magnitude to the labile N pools, supporting their potential use to make more accurate fertilizer recommendations (Haney et al., 2018).

In this experiment the farmers did not adjust N fertilizer rates based on the soil N pools, but the nitrogen balances were all negative. As a result of the poor correlation with PMN and nitrogen balance, it is possible that other factors limited the yield. In these cases, the same yield would have also been achieved with lower N fertilizer levels as in (Haney et al., 2018). The relatively high ISNT-N amounts in the soil would indicate that many of the studied soils would have a low response to N fertilizer, as in (Khan et al., 2001). Further work testing variable rate or plant N concentration based N fertilizer applications could evaluate the potential of PMN to predict soil N supply further (Tremblay et al., 2008).

The soil N pools were unevenly distributed (stratified) in the soil profile. Topsoil contained ca. 61%–63% of the more slowly cycling N pools and 66%–72% of the more labile nitrogen pools (WSON and PMN). This indicates that basing the PMN estimate only on topsoil samples underestimates the N supply to crops by ca. 30%. However, our sampling depth was only to 30 cm and a deeper sample could have found more labile N in the subsoil profiles. There was no difference between the stratification of N pools between treatment and control plots, which did not support using the stratification of OM as a soil health metric (Franzluebbers, 2002). At least the 4-year experiment did not result in a detectable overall effect on the stratification of N pools between 0 and 17 cm versus 18 and 31 cm depth profiles.

It has been suggested that carbon sequestration would need additional N fertilization to balance the N bound up by increasing organic matter stocks (Rumpel et al., 2019). Organic matter is often assumed to have a 10:1 C:N ratio, but it is highly variable ranging from 8 to 25:1 depending on the nutrient status and degree of decomposition (Tipping et al., 2016). The C:N ratio of the studied soils was 14 ± 3:1, placing them in closer to nutrient-rich soils (Tipping et al., 2016). The C:N ratio of carbon farming soils was slightly, but significantly smaller than those of the current practise control soils (2% decrease, p = .02). This decrease did not support the idea of carbon additions resulting in a higher C:N ratio. In contrast, carbon farming practises that include legumes (van der Pol et al., 2022) or recycle otherwise leachable N (Aronsson et al., 2016) seem to build labile N pools and increase N mineralisation to crop plants.

The water-soluble C:N ratio (WSOC:WSON) was 30% higher than the total soil C:N (average 18 ± 6:1). Only about 32% of the studied soils had a lower WSOC:WSON than C:N and the correlation between water-soluble and total pools was very weak (R^2 = .01 and .05 on control and treatment plots, respectively). This supports earlier work, which indicates that for microbial N mineralisation, the water soluble C:N ratio is more important than bulk soil C:N (Haney et al., 2012). There was no consistent trend for carbon farming increasing the WSOC:WSON ratio. Individual farms had differences in the ratios of 50%–119%, but the variation was so great even within management action groups, that no statistical significance was found. The higher C:N ratio in the water-soluble pool than in the bulk soil can indicate a fresh input of carbon from plant residues or strong immobilization of water-soluble N to mineral matter (Daly et al., 2021). A time series of the C:N ratio in litter, particulate organic matter, water-soluble phase and mineral-associated organic matter would be valuable research to explore the dynamics of C:N following carbon inputs to soil. The more recent C cycle models, which include labile N pools can be a valuable tool in this

(Zhang et al., 2021). Labelling plant inputs with stable ^{15}N isotopes could help track the flow of nitrogen in this kind of time series study (Putz et al., 2011).

The farmers of this experiment did not reduce the fertilization based on soil N supply, as they did not have the information available when planning fertilization. Other studies have found, that if PMN forecasts are used, fertilizer application can be reduced 30%–50% while maintaining yields (Harmel & Haney, 2013). As the response of PMN to different management actions is highly variable (Table 2), and it does not depend on the soil carbon and clay levels, providing general N credits from cover crops and soil types does not seem feasible. Instead, measuring one or more labile N pools can provide valuable information for increasing fertilizer use efficiencies.

4 | CONCLUSIONS

Soil contains large amounts of organic nitrogen in pools of differing availability for plants. We investigated the size of some of these pools in 40 fields on 20 farms, in both top- and subsoil to evaluate if they change with management and if they can be used to improve fertilization practices. The crops removed more N than what was supplied from fertilizer or manure, indicating that ca. 30% of the N in the harvested crop came from bioavailable soil N reserves. As the farmers did not adjust their fertilization based on the forecasted N release (PMN), and other limitations to yield, the N balance did not correlate well with PMN. Management over the 4-year experiment duration had resulted in small increases in ISNT-N pools and a moderate increase in the PMN mineralisation rate. Pools such as ACE protein, total N and WSON were too variable to draw conclusions over several farms and soil properties covered in this experiment.

The largest organic N pools (ISNT-N, total N and ACE protein) were largely controlled by slowly changing or permanent soil properties, such as clay %, carbon content and C:clay-ratio. As the clay content increased, ISNT (potentially of bacterial origin), but protein N (potentially from fungal origin) decreased, highlighting the strong and differing effect of soil texture on the biological cycling of N. Contrary to previous studies, the protein and ISNT pools comprised only 11%–13% of the total N, leaving a large fraction of the total N unidentified. The unidentified fraction also correlated strongly with clay and C, suggesting that it might be linked to tightly bound mineral-associated organic matter (MAOM). Overall, the unidentified pool may consist of several tonnes of N per hectare, or enough to supply N for plants for 60 years. Of the investigated pools, especially the ISNT pool and CO_2 burst-based potentially mineralized N seem promising, as they respond to management and represent slowly and rapidly cycling N pools. Overall, our findings highlight the importance of measuring soil bioavailable N pools to build an overall picture of the short and long-term N mineralisation potential of individual agricultural fields.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at https://zenodo.org/communities/carbonaction/.

ORCID

Tuomas J. Mattila https://orcid.org/0000-0002-3369-8623

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