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Original Article

Carbon to nitrogen ratio and quantity of organic amendment interactively affect crop growth and soil mineral N retention

Maartje van der Sloot[a,*], David Kleijn[a], Gerlinde B. De Deyn[b], Juul Limpens[a]

[a] Plant Ecology and Nature Conservation Group, Wageningen University, Droevendaalsesteeg 3a, 6708PB, Wageningen, the Netherlands
[b] Soil Biology Group, Wageningen University, Droevendaalsesteeg 3a, 6708PB, Wageningen, the Netherlands

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ABSTRACT

Using organic amendments to improve arable soils in the long term is a careful balancing act of applying amendments with the right carbon to nitrogen ratio (C:N ratio) at adequate quantity to avoid nitrogen (N) leaching while promoting or retaining crop growth in the short term. So far, most studies examining the relationship between C:N ratio and N mineralization and immobilization were done without plants. In this study we explored how crop biomass and N leaching change with increasing C:N ratio and quantity of organic amendments to arable soil. We conducted an open-air mesocosm experiment with organic amendments application across a range in C:N ratio (10 to 60) and quantity (10 to 50 ton ha^{-1}) to sandy arable soil using a full-factorial design. Spring wheat was planted and grown for six months during which three rainfall events were simulated to test treatment effects on N leaching. Applying amendments with a C:N ratio of 20 and higher decreased crop biomass and increased mineral soil N, while amendments with a C:N ratio of 10 had the opposite effect. Applying larger quantities of amendments reinforced the effect of the C:N ratio on crop biomass. N leaching remained unaffected by either amendment C:N ratio or quantity or even mineral fertilizer as N leaching only occurred in the control treatment without plants. Our results suggests that growing a crop is adequate to prevent N leaching. Applying organic amendments do not pose a different risk regarding N leaching when compared to mineral fertilizer and slurry.

1. Introduction

Meeting the increasing global food demand while minimizing the impact on the environment is a major challenge in crop production in the next decades (Arneth et al., 2019; Godfray et al., 2010). Sustainable soil management is an important factor when reaching these future food production goals (Lal, 2010; Tilman et al., 2011). Increasing Soil Organic Matter (SOM) content can alleviate environmental problems in sandy arable soil such as nitrogen (N) leaching while promoting crop growth and the soil microbiome (Arneth et al., 2019; Diacono and Montemurro, 2011; EEA, 2005; Lal, 2006; Tester, 1990; Wei et al., 2016). To maintain and even increase SOM content, annually amending arable soil with organic material is a time-tested technique (Freibauer et al., 2004; Lal, 2006; Wei et al., 2016). This has inspired studies on the impact of organic amendments on arable soil quality and associated soil functions over the last decade (Diacono and Montemurro, 2011; Eden et al., 2017; Wei et al., 2016). These studies suggest that major effects on production related processes, primarily crop growth and mineral N leaching, depend on the quantity of the organic material that is being added but also the quality.

The quality of the organic amendments is largely determined by the carbon to nitrogen ratio (C:N ratio) as it relates to how fast the applied organic N will become plant available as mineral N. Upon decomposition of the organic amendments, soil microorganisms use N for enzyme production and to grow which can lead to temporal N immobilization in microbial biomass when the C:N ratio of the organic material is too high. To be able to break down low quality organic amendments, such as straw that has a C:N ratio of approximately 100, the soil microbes need all the N that is present in the organic material. Additionally the soil microbes will scavenge N from the soil solution that becomes mineralized from the soil inherent SOM, leaving little or no freely available N in the soil (i.e. net N immobilization (Flavel and Murphy, 2006; Hadas et al., 2004; Nicolardot et al., 2001; Sikora et al., 2001)). High quality amendments, such as composts that have a C:N ratio of approximately 10, contain relatively more N than the soil microbes need during decomposition and will therefore increase soil mineral N availability (i.e. net N mineralization

* Corresponding author.
E-mail address: maartje.vandersloot@wur.nl (M. van der Sloot).

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(Flavel and Murphy, 2006; Hadas et al., 2004; Nicolardot et al., 2001; Sikora et al., 2001)).

How amendment C:N ratio is related to mineralization or immobilization has been explored by Nicolardot et al. (2001) using a simple dynamic model that predicts N availability in the soil after application of crop residue with different C:N ratios. The model was validated using several crop residues that ranged in quality from rye plants (C:N ratio of 9.5) to wheat straw (C:N ratio of 139). The model predicted net N immobilization at C:N ratios above 25 and net N mineralization at C:N ratios below 15. These predictions were subsequently corroborated empirically by Kaleeem Abbasi et al. (2015) who found immediate net N mineralization for residues with C:N ratios of 12.7, 14.4 and 26.4, immediate net N immobilization for residues with C:N ratios of 36.4, 49.2 and 121.5, and initial N immobilization followed by N mineralization after 120 days of incubation with residues with C:N ratios of 12.1 and 20.9.

Enhancing SOM content by means of application of organic amendments to soil such that it does not reduce crop growth or increase N leaching requires a better understanding of how the quantity and quality of organic amendments influence these processes (Andersen and Jensen, 2001; Murphy et al., 1998; Steen Jensen and Ambus, 1999). Applying large quantities of organic material with a low C:N ratio would ensure the mineralization of sufficient N to maximize crop growth (Flavel and Murphy, 2006; Steen Jensen and Ambus, 1999) but may increase harmful N leaching (Malcolm et al., 2019; Steen Jensen and Ambus, 1999). Whether leaching occurs depends on how much of the mobile N (mainly nitrate) is being taken up by the roots of the growing crop which would prevent it from flushing to the groundwater during heavy rainfall. Organic amendments with a high C:N ratio can generally reduce the risk of N leaching due to N immobilization (Bergström and Kirchmann, 2004; Malcolm et al., 2019). However, this can trade-off with reduced crop growth. Using organic amendments to improve arable soils in the short and long term is therefore a careful balancing act of applying amendments with the right C:N ratio at adequate quantity to avoid N leaching while supporting crop growth. So far, most studies examining the relationship between C:N ratio and N mineralization and immobilization were done without crop growth (Kaleeem Abbasi et al., 2015; Nicolardot et al., 2001; Vigil and Kissel, 1991). Therefore, it is still warranted to test the impact of organic amendment C:N ratio and quantity on N leaching and crop biomass when the crop is seeded shortly after incorporation of the organic amendments into the soil.

In this study we set-up an open-air mesocosm experiment to examine the relationship between organic amendments quality (C:N ratio) and quantity on crop biomass, N leaching and soil N retention. Our main objective was to investigate how crop biomass, N leaching and soil N retention change with increasing quantity and C:N ratio of organic amendments on sandy arable soil. We hypothesized that: (1) with increasing C:N ratio, crop biomass and N leaching would be lower and soil N retention will be higher; (2) applying a higher quantity of organic amendment would partly offset the effect of C:N ratio; and (3) crop biomass, N leaching and soil N retention would be more affected by organic amendment C:N ratio than by its quantity.

2. Materials and methods

To examine the effects of C:N ratio and quantity of organic amendments on crop biomass, N leaching, and soil N retention, we performed a mesocosm experiment from January 2020 until June 2020. To avoid the confounding effect of C:N ratio and type of organic material applied (Flavel and Murphy, 2006; Hadas et al., 2004; Huang et al., 2004), we created organic amendments with different C:N ratios using a single source of organic material (plant cuttings) in combination with cow slurry (C:N ratio 7.3) to lower the C:N ratio or mixed with dried straw (C:N ratio 310.7) to raise the C:N ratio. We used the C:N ratio rather than lignin: N ratio as proxy for organic amendment quality (Becker et al., 1994; Kumar and Goh, 2003; Taylor et al., 1989) for practical reasons. The C:N ratio is easier to measure for most labs and, given our very different source materials (road verge cuttings, straw, and slurry) sufficient for creating a range in degradability (Nicolardot et al., 2001; Taylor et al., 1989) wide enough to test our hypotheses. The experiment comprised a full factorial combination of six C:N ratios (10, 20, 30, 40, 50, 60) and three amendment quantities (the equivalent of 10, 30, and 50 ton fresh weight ha^{-1}) with each treatment being replicated five times. All mesocosms were placed in a randomized block design (five blocks of 26 mesocosms each). Mesocosms were placed outside under a transparent roof with open sides. Climate conditions during the experiment were similar to other years in this period in the Netherlands (KNMI, 2020). The water regime entailed watering twice a week by adding roughly 250 ml of water to all mesocosms during the whole duration of the experiment. This was less than average rainfall in these months in the Netherlands (equivalent of 1.5 L per mesocosm) but this was necessary to prevent intermediate N leaching outside the rainfall events. The regular watering together with the rainfall events were estimated to be sufficient for crop growth.

2.1. Soil collection

Sandy arable soil was collected from an arable field in the vicinity of Wageningen (51°59′28.9″N, 5°39′31.5″E; The Netherlands). The soil had 3.16 ± 0.03% organic matter, a pH of 7.39 ± 0.05, and mineral N and phosphorous content were 6.81 ± 0.46 and 1.33 ± 0.09 mg kg^{-1}, respectively. This soil was air dried and mixed in a cement mixer with water to gravimetrically result with an average moisture level of 16.75 ± 0.06% in fresh weight. Cylindric mesocosms (40 cm high, diameter 20 cm) were filled with soil (bulk density of 1.22 ± 0.01 g cm^{-3} based on dry weight). The open bottom of the mesocosms was covered with root cloth and were standing on individual trays to allow both free leaching of excess water and collection of leachate.

2.2. Treatments

Organic amendment treatments were prepared from herbaceous road verge cuttings collected in September 2019 from public road verges in the municipality of Sint Anthonis (51°37′33″N, 5°52′52″E; The Netherlands). After storing the material at 4 °C for four months, these cuttings were subsequently mixed with either cow slurry (C:N 7.3) or wheat straw mulch (C:N 310.7) to create the six C:N ratio treatments. All C:N ratio calculations are based on amount of C and N in fresh weight of the material. Moisture percentages of the start materials are provided in the appendix (Appendix A, Table 1). Each C:N ratio treatment was applied at three quantities resulting in eighteen organic amendment treatments (Appendix A, Table 1). Each organic amendment treatment was mixed in with the upper 10 cm of the soil, in line with agricultural practice. Ten days after mixing in the organic amendments, the mesocosms were seeded with spring wheat (*Triticum aestivum,* Harenda variety Agrifirm). Fifteen seeds were placed in each mesocosm and just after sprouting, the plant density was reduced to 10 viable plants per mesocosm, in line with regular density in arable fields (275 to 300 seedlings m^{-2}).

To compare the effect of organic amendments to conventional fertilizer application practices, we included a cow slurry-only treatment in our experimental design comparable to regular amounts of fertilization during this six-month period (80 kg N ha^{-1}) for spring wheat in the Netherlands. We also applied three levels of mineral fertilizer (Limestone Ammonium Nitrate; 24% N of 50-50% nitrate-ammonium at the levels 21, 80 and 164 of kg N ha^{-1}). Slurry and mineral fertilizer treatments were added directly on top of the mesocosms just after crop emergence to mimic conventional farming methods. To assess the crop nutrient uptake from N mineralized from the soil organic matter during the experiment, a control treatment was added without fertilization. To quantify N leaching from the soil in absence of a crop a last control without plants and without fertilization was added. Chemical composition of all treatments are included in table 1 in appendix A. 250 ml of a nutrient solution (2.61 g L^{-1} K_2SO_4; 2.46 g L^{-1} $MgSO_4{\cdot}7H_2O$; 1.47 g L^{-1} $CaCl_2{\cdot}2H_2O$) was applied to all mesocosms (except the no fertilizer control) three weeks after crop emergence to prevent effects frommicronutrients deficiency during crop growth.

2.3. N leaching

Three heavy rainfall events were simulated during the experiment. The simulations were six weeks apart and took place the day after regular watering to have sufficient soil moisture for leaching. The amount of water applied was based on data from a nearby weather station (KNMI weather station 583, Wageningen, The Netherlands, (KNMI, 2020)). Data from 1989 to 2019 showed a monthly rainfall of 20 mm (which is 20 L rain over 1 m^2). For our mesocosms experiment this is the equivalent of 750 ml water per mesocosm, which we applied at the top of the mesocosms at a rate of 250 ml water h^{-1} over a period of 3 h. The day after a rainfall simulation the leachate was collected by taking the water out the collection trays with a syringe. Total amounts of the leachate was weighed to calculate leachate volume. The total weight of the leached water was used to calculate the percentage of water retention, i.e. the percentage of rainwater that can be retained by arable soil in the crop-soil layer. Percentage of water retention in this study was calculated as the amount of water not leached out (thus retained) as a percentage of the total amount of water applied (i.e. 750 ml per rainfall event). A subsample of 20 ml leachate was stored overnight in a freezer at −20 °C prior to chemical analysis the next day.

2.4. Data collection

2.4.1. Crop variables

Total aboveground biomass of the wheat plants was determined just before seed maturation after six months of growth when the plants were at stage GS61 according to Zadok's scale (Zadoks et al., 1974). Shoots were cut off at the soil surface and were dried at 70 °C for three days and then weighed to determine the shoot biomass dry weight.

2.4.2. Mineral soil N concentration

Five soil cores per mesocosm were taken at the end of the experiment using a 2-cm diameter soil auger. Per core the 0–10 and 20–30 cm layer were collected separately, and per mesocosm the soil of the five cores was pooled per layer and stored in zip-lock bags at −4 °C until further analysis. Roots were sieved out over a 2 mm sieve and soil was dried at 40 °C prior to the soil chemical analysis. To assess the plant available N, mineral N (NO_3–N, NH_4–N) concentration in the soil was analysed for both soil layers. Soil mineral N extraction was performed according to standard procedures (Temminghoff, 2010) using a 1:10 (*w:v*) ratio of dried soil: 0.01 mol L^{-1} $CaCl_2$ solution at 20 °C. The concentration of mineral N in the extracts was analysed with a segmented-flow analyser (Skalar San++ system). Separate soil layers did not show significant differences in soil mineral N concentration and were therefore pooled for further analyses. These pooled mineral N measurements were treated as the legacy soil mineral N retention.

2.4.3. Leachate analyses

All leachate samples were analysed the day after collection. Mineral N (NO_3–N, NH_4–N) concentration was determined with a continuous flow analyser (Temminghoff, 2010) (Skalar San++ system). The total amount of mineral N leached was calculated by multiplying the concentrations of both NO_3–N and NH_4–N by the volume leached. The cumulative N leached per mesocosm over three leaching events was summed to create the parameter 'N leached'.

2.5. Statistical analyses

All statistical analyses were performed in R studio version 2021.09.2+382 with core R version 4.1.2 (R Core Team, 2013). To explore relative importance of direct and indirect effects of organic amendment C:N ratio and quantity on crop biomass, N leaching, and soil N retention, we used Structural Equation Modelling (SEM; R package 'piecewiseSEM' (Lefcheck, 2016; R Core Team, 2013)) according to an a priori conceptual SEM model we developed (Fig. 1). To test this SEM model, we only used data from the organic amendment treatments (so excluding the control, slurry and mineral fertilizer addition treatments). The C:N ratio and quantity of the organic amendments were considered the exogenous variables. The endogenous dependent variables included in the model were crop biomass (crop biomass; total aboveground crop biomass in g dry weight per mesocosm), percentage moisture retained in the soil after the rainfall events (percentage of water retained; calculated by the percentage of water volume retained after a rainfall event), cumulative amount of mineral N leached over all three rainfall simulations (N leached; in mg mineral N per mesocosm) and the concentration of mineral N in the whole soil column at the end of the experiment (legacy soil mineral N; in mg N kg^{-1} soil) after log transformations to achieve normality and standardization. Block location was added as a random factor. The Fisher's Chi-square test was used to test goodness of fit of the model (Lefcheck, 2016). The goodness of fit of this SEM entails $0 \leq \chi^2$, df ≤ 2 and $P > 0.05$.

To compare the effect of organic amendment application on crop biomass, N leaching and soil N retention with mineral and slurry fertilization methods, we used linear mixed effects models (R package 'lmerTest' (Kuznetsova et al., 2015)). The model tested the effect of the different fertilization methods (organic amendment, slurry, mineral fertilizer low, regular and high level) and controls without fertilization (control with and without plants) as fixed independent variables on the dependent variables of crop biomass, N leaching and soil N retention which were log transformed to meet the requirement of a normal distribution. Block was added as random factor. Subsequently, one-way ANOVA's and Post-hoc Tukey tests were used to determine the relative effects of the treatments. All significance levels were assessed at $P < 0.05$.

Finally, linear mixed effects models were used to examine whether the C:N ratio influences the relationship between the quantity of the applied N and crop biomass, N leaching and soil N retention. For these analyses, data from the organic amendment treatments (C:N ratio as numeric variable) and the mineral fertilizer treatments (C:N ratio as zero) were used. The amount of N applied and the C:N ratio, as well as their interaction were included as independent variables and log transformed crop biomass, N leaching and soil N retention data as dependent variables and block was added as random factor. Relations between dependent and

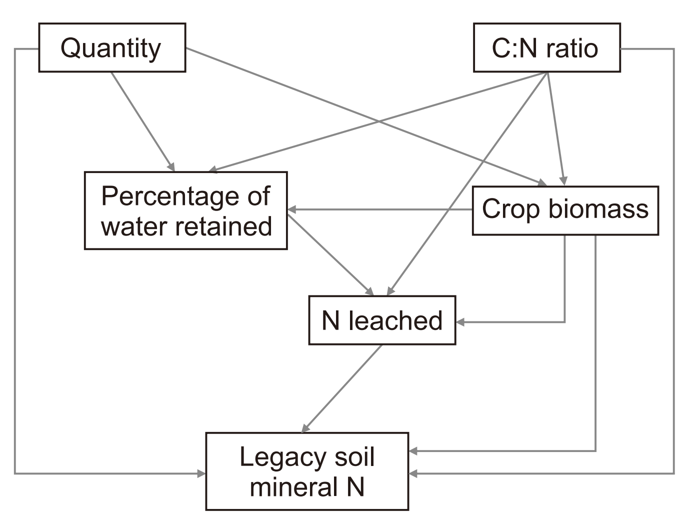

Fig. 1. Conceptual Structural Equation Model (SEM) showing the expected effects of organic amendment C:N ratio and quantity on crop biomass, N leaching and soil N retention.

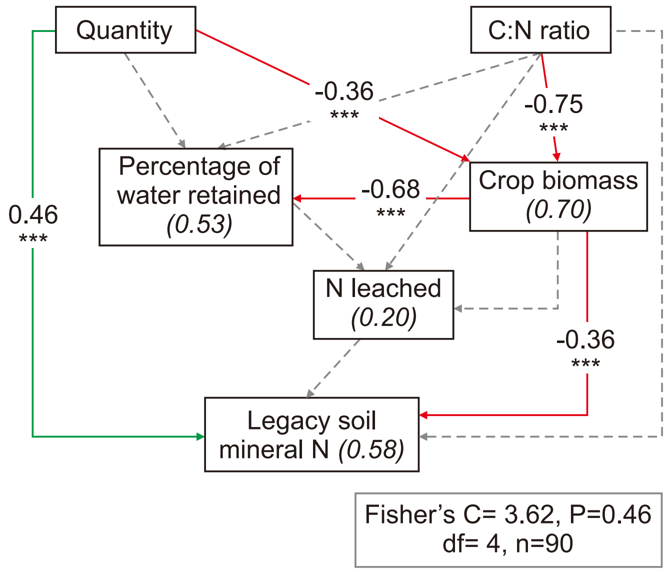

Fig. 2. Piecewise Structural Equation Model (SEM) of the effect of the C:N ratio and quantity of an organic amendment on crop biomass, the percentage of water retained, N leached and legacy soil mineral N. Green arrows show a significant positive path, red arrows show a significant negative path and grey and dashed arrows show a non-significant path. Numbers on the significant arrows indicate standardized path coefficients and the strength of the path coefficient is shown proportionally to the width of the arrow. The proportion of the variation explained by the fixed and random predictor variables (conditional R^2) is shown as the number between brackets in the box of each response variable. The grey box in the bottom of the figure shows the result of the Fisher's exact test (Fisher's C), P value (P) of the test, degrees of freedom (df) of the model and the number of observations (n) used for the analysis.
$^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

independent variables (and their interaction) were tested with t-statistics using Satterthwaite's method (Satterthwaite, 1946) to calculate the degrees of freedom. Significant relations ($P < 0.05$) are reported in the results section.

3. Results

3.1. Effects of C:N ratio and quantity of organic amendments

Crop biomass was negatively related with both C:N ratio and quantity of the organic amendments, but these amendment properties had limited impact on N leaching (Fig. 2). The percentage of water that was retained in the soil after the rainfall events was lower with higher crop biomass. The amount of mineral N left in the soil at the end of the experiment increased with increasing amount of organic amendments applied and was not directly affected by the C:N ratio of the organic amendments, only indirectly through its impact on crop biomass.

3.2. Organic amendments versus slurry and mineral fertilization methods

On average, the addition of organic amendments resulted in a significantly lower crop biomass compared to all mineral fertilizer and slurry treatments (Fig. 3A; F-test 24.4, P value < 0.01). On average, and across all organic amendment treatments with different C:N ratios, even the unfertilized plants (control +) produced more crop biomass than those growing in mesocosms with organic amendments. N leaching was not significantly different between the application of organic amendments (cuttings) or all levels of mineral fertilizer and slurry (Fig. 3B; F-test 9.1, P value < 0.01). Only the control treatment without plants (control -) differed significantly in N leaching with approximately 10 times more N leached than the average of all other treatments. Despite the significant N leaching losses, there was a large amount of mineral N left in the soil at the end of the experiment in the mesocosms with the control treatment without plants (Fig. 3C; F-test 10.5, P$<$ 0.01). Adding organic amendments resulted in significantly higher amounts of legacy mineral N at the end of the experiment compared to the high and regular mineral fertilization treatments and the slurry treatment.

The relationship between the quantity of the applied N and crop biomass was strongly dependent on the C:N ratio of the amendment (Fig. 4A; significant interaction between C:N ratio and the amount of N applied). Application of mineral fertilizers or organic material with a C:N ratio of 10 generally resulted in a positive relationship between the amount of N applied and crop biomass. Application of organic amendments with C:N ratios of 20 or higher resulted in negative relationships between the amount of N applied and crop biomass. The amount of N applied or the C:N ratio did not influence N leaching in any of the treatments (Fig. 4B). The relationship between the quantity of the applied N and amount of mineral N remaining in the soil after crop harvest was influenced by the C:N ratio of the applied treatments (Fig. 4C; significant interaction between C:N ratio and the amount for N applied). In the mesocosms with mineral fertilizer treatments, the mineral N that was left in the soil decreased with increasing N application. Applying more N through organic amendments generally resulted in higher legacy mineral N, which furthermore consistently increased with increasing C:N ratio.

4. Discussion

The objective of this study was to investigate how crop biomass, N leaching and soil N retention change with increasing quantity and C:N ratio of organic amendments on sandy arable soil. We found that application of organic amendments with a C:N ratio of 20 or higher decreased crop biomass (Figs. 2, 3A and 4A) and increased mineral N retention in the soil (Figs. 3C and 4C). The amount of mineral N retained was directly and positively affected by the quantity of organic amendment applied and indirectly and positively affected by the C:N ratio through crop biomass (Fig. 2). Contrary to our hypotheses, the cumulative amount of mineral N that leached after simulations of heavy rainfalls was not affected by the organic amendments C:N ratio, quantity applied or by separate mineral fertilizer or slurry application (Figs. 2, 3B and 4B). Significant N leaching was only observed when there was no crop present to take up mineral N (Fig. 3B).

4.1. Crop biomass growth

Crop biomass decreased with increasing C:N ratio and quantity of the organic amendment (Fig. 2), as hypothesized. Specifically we observed a decline in crop biomass compared to mineral fertilizer when the C:N ratio was 20 or higher (Fig. 4A) probably because mineral N was immobilized. N immobilization is likely, given that the crop in the unfertilized control grew significantly more biomass than the mean crop biomass across all amendment treatments (Fig. 3A). When there was an organic amendment applied with a C:N ratio of 10, the N in the amendment was readily available, and crop biomass did not significantly differ from adding a similar amount of N as mineral fertilizer. Increasing the amount of organic amendment applied reinforced the impact of C:N ratio on crop biomass, decreasing crop biomass when applying amendments with a C:N ratio above 20 and increasing crop biomass when applying a C:N ratio of 10. Previous studies (Kaleeem Abbasi et al., 2015; Nicolardot et al., 2001) found similar tipping points between net N mineralization and immobilization when measuring and modelling N dynamics in the soil. However, these studies did not include the effect on crop biomass. Our study shows that the crop biomass response closely follows the moment when N mineralization starts to dominate over N immobilization, illustrating the strong microbial control on crop growth in N limited conditions.

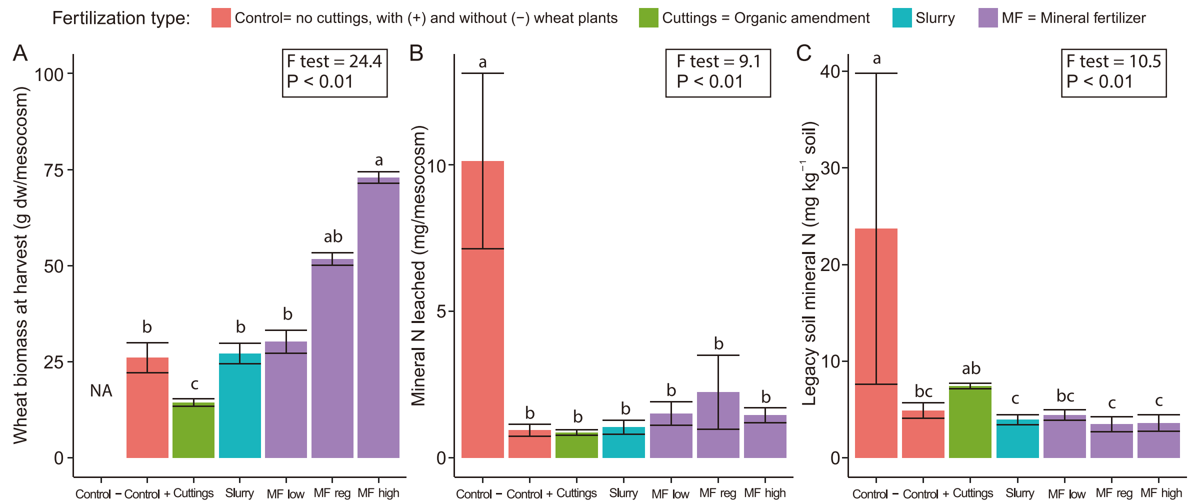

Fig. 3. Fertilization treatment effects on crop biomass (A), amount N leached (B) and legacy soil mineral N (C). Error bars indicate standard error of the mean (Control - n = 6; Control + n = 6; Cuttings n = 90; Slurry n = 7; MF low n = 7; MF reg n = 7; MF high n = 7). Letters indicate significant differences between treatments at P < 0.05 tested with a one-way ANOVA and post-hoc Tukey HSD test. The grey box shows the result of the F test and corresponding P value (P) of the ANOVA.

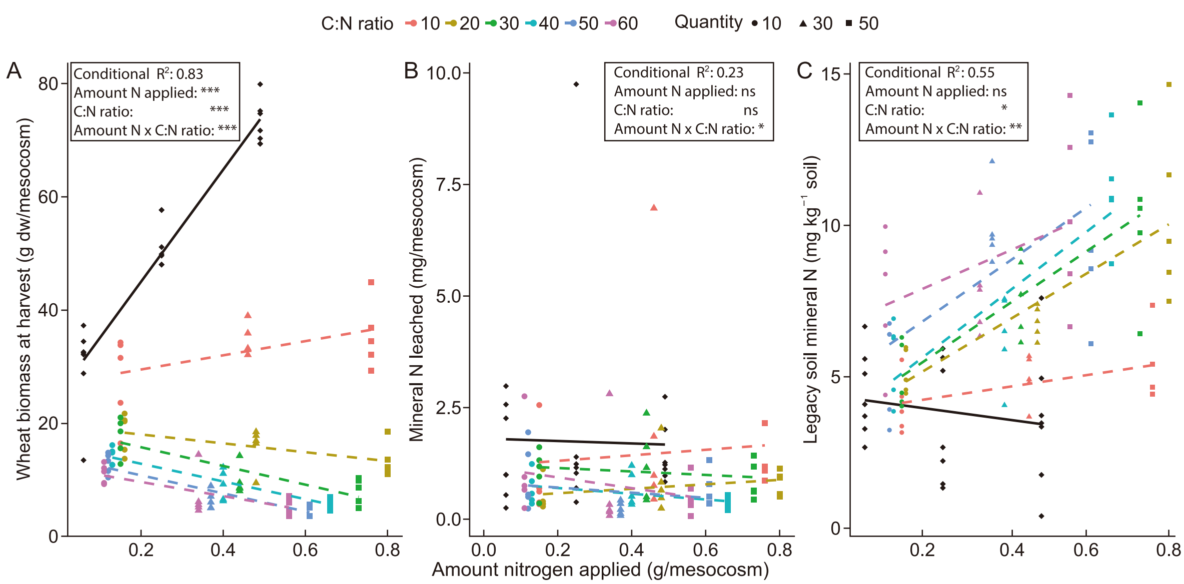

Fig. 4. Crop biomass (A), amount N leached (B) and legacy soil mineral N (C) in relation to the amount of nitrogen applied with the organic amendment treatments (coloured points) and mineral fertilizer treatments (black points). Correlations significance is analysed using linear mixed effect models including C:N ratio and amount of nitrogen applied as independent variables and their interaction (P < 0.05). Results of conditional R^2 values and correlations of the models are presented in grey boxes.
*P < 0.05, **P < 0.01, ***P < 0.001.

4.2. N leaching

N leaching was not affected by the organic amendments application quantity or C:N ratios (Figs. 2, 3B and 4B). Even the highest amount of N applied via mineral fertilizer did not result in mineral N leaching concluding no effect from the fertilization treatments (Figs. 3B and 4B). Leaching was, however, significantly higher in the control treatment without crop growth. This suggests that N uptake associated with growth of the wheat plants was sufficient to prevent N leaching during our experiment. Our results are in line with the N capture capacity of cover crops during fallow periods: it has been shown that cover crops can reduce nitrate leaching on average by 56% during the main crop growth (Thapa et al., 2018). While the presence of a crop prevented N leaching from our experimental mesocosms even in the mineral fertilizer treatments, the scavenging for N by crops is possibly not strong enough to prevent N leaching during periods of low crop cover in winter during heavy rainfall events or during fallow periods between crops. While organic amendments with high C:N (above 20) may help preventing leaching in the short term during the winter period by microbial N immobilization, they could also prevent leaching by improving water retention in the longer term as the SOM content increases with organic amendment application (Tester, 1990). Extrapolating these results to the field situation comes with a note of caution. Actual N leaching in the field strongly depends on both local soil, crop and weather conditions which are not captured in our experiment. Next to that, the timing of N availability, as has been shown to be difficult to forecast when applying organic amendments, is important when extrapolating the findings from this experiment regarding N leaching to field level. Measuring N leaching in a field experiment over a whole growing season including a fallow period with and without organic amendment application will help in further understanding the potential of organic amendments to decreasing excess N leaching.

4.3. Soil mineral N retention

The amount of mineral N retained at the end of the experiment was directly driven by the quantity of the organic amendment and indirectly by the C:N ratio. Applying a larger quantity of organic amendment increased the amount of mineral N retained at the end of the experiment and this was most apparent when applying organic amendments with a C:N ratio of 20 or more (Figs. 2, 3C and 4C). These results suggest that the N released from the organic amendment was initially immobilized, while being mineralized towards the end of the experiment. Kaleeem et al. (2015) also saw this result after applying organic amendments with C:N ratios of 12.1 and 20.9 with initial immobilization and mineralization afterwards. Lazicki et al. (2020) showed in an incubation experiment of 84 days at 23 °C that 0% N had mineralized from organic amendments with a C:N ratio of 20 whereas from amendments with C:N ratio of 6 about 30% of the added N became mineralized. However, in our SEM analysis (Fig. 2) we observed that leftover mineral N in the soil was directly affected by the quantity applied but not directly by the C:N ratio as this parameter only worked indirectly through effects on crop biomass. The studies of Kaleeem et al. (2015) and Lazicki et al. (2020) both did not include the effect of crop growth in their experiments which can explain this difference. Despite adding high quantities of amendment, N availability did not line up with N requirement from the wheat during our experiment. This result implies that the timing between the organic amendment application and the peak N demand of the crop was not optimally aligned in our experiment and illustrates the difficulty of timing soil processes to crop growth.

4.4. Implications of this study

In this study we confirmed already known tipping points of N mineralization and immobilization (Flavel and Murphy, 2006; Hadas et al., 2004; Kaleeem Abbasi et al., 2015; Nicolardot et al., 2001; Sikora et al., 2001; Vigil and Kissel, 1991). However, our study provided more understanding in linking the results of these mechanisms towards practical measures such as crop growth and N leaching. Overall we confirmed part of our first hypothesis that increasing C:N ratio results in low crop biomass and indirectly increased soil mineral N retention. Especially C:N ratios above 20 will result in N immobilization which limits the crop growth when organic amendment is applied directly before sowing. This is a problem when convincing farmers to use organic amendments as soil improver since decreased crop yield will result in decreased income. Applying more N with the organic amendments would not fix this problem, unless amendments with C:N ratio of 10 or lower are used, because we showed that applying a larger quantity will merely decrease crop growth even more when the crop is seeded shortly after the application of the organic amendment to the soil. In our second hypothesis we suspected that adding a larger quantity will offset the effect of C:N ratio, however this was contradicted by the results discussed. Our third hypothesis is therefore true for crop growth since the C:N ratio is largely dictating the effect on crop biomass. N leaching was not affected by the different organic amendment treatments or fertilization treatments and therefore we cannot confirm our hypothesis of the effect of different C:N ratio or quantity on N leaching. In practice this entails that, according to our study, the addition of organic amendments (even with a low C:N ratio) has a similar low risk of N leaching as mineral fertilisers when measured during the cropping season. The next step in safely using organic amendments in agriculture is taking previous knowledge of the underlying mechanisms combined with our findings and perform a large scale field experiment over multiple crop cycles where one can investigate if the results regarding crop growth, N leaching and soil N retention remain similar.

5. Conclusions

We investigated the effect of differing C:N ratio and quantity of organic amendment application on crop biomass, N leaching an soil N retention and compared this with conventional fertilizers such as mineral fertilizers and slurry. Our results show a decrease in crop biomass and an increase in mineral soil N retention after application of an organic amendment with a C:N ratio 20 and higher. This shows the importance of balancing the timing of application of an organic amendment and the C:N ratio to meet the goal in providing sufficient crop growth while over the long term improving agricultural soil. N leaching was only observed when there were no plants present indicating no severe risk of N leaching during crop growth for both organic amendment, mineral fertilizer and slurry application. A large scale field study is the next step that should test if field impact of organic amendments on crop biomass and soil N leaching match the results of our pot experiment.

Authors' contributions

MvdS co-designed the experiment, conducted the experiment and drafted the manuscript. DK, GDD, and JL co-designed the experiment and revised the manuscript. The authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

C	carbon
N	nitrogen
C:N	carbon to nitrogen ratio
SOM	soil organic matter content
SEM	structural equation model

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crope.2022.08.001.

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