



RESEARCH ARTICLE

Strategies for high nitrogen production and fertilizer value of plant-based fertilizers

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This article has been edited by Klaus Dittert.

Abstract

Background: Organic vegetable production has a demand for alternative fertilizers to replace fertilizers from sources that are not organic, that is, typically animal-based ones from conventional farming.

Aims: The aim of this study was to develop production strategies of plant-based fertilizers to maximize cumulative nitrogen (N) production (equal to N yield by green manure crops), while maintaining a low carbon-to-nitrogen (C:N) ratio, and to test the fertilizer value in organic vegetable production.

Methods: The plant-based fertilizers consisted of the perennial green manure crops—alfalfa, white clover, red clover, and a mixture of red clover and ryegrass—and the annual green-manure crops—broad bean, lupine, and pea. The crops were cut several times at different developmental stages. The harvested crops were used fresh or pelleted as fertilizers for field-grown white cabbage and leek. The fertilizer value was tested with respect to biomass, N offtake, N recovery, and soil mineral N (N_{\min}). Poultry manure and an unfertilized treatment were used as controls.

Results: The cumulative N production of the perennial green manure crops ranged from 300 to 640 kg N ha⁻¹ year⁻¹ when cut two to five times. The highest productions occurred at early and intermediate developmental stages, when cut three to four times. Annual green manure crops produced 110–320 kg N ha⁻¹ year⁻¹, since repeated cutting was restricted. The C:N ratio of the green manure crops was 8.5–20.5, and increased with developmental stage. The fertilizer value of green manure, as measured in white cabbage and leek, was comparable to animal-based manure on the condition that the C:N ratio was low (<18). N recovery was 20%–49% for green manure and 29%–42% for poultry manure. A positive correlation was detected between soil N_{\min} and vegetable N offtake shortly after incorporating the green manure crops, indicating synchrony between N release and crop demand.

Conclusions: Plant-based fertilizers represent highly productive and efficient fertilizers that can substitute conventional animal-based fertilizers in organic vegetable production.

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KEYWORDS

C:N ratio, cut-and-carry, green manure, nitrogen, vegetables

1 | INTRODUCTION

In organic plant production, fertilizers from conventional farming (e.g., animal-based manure and slaughterhouse by-products) may be used when nutritional needs cannot be met by fertilizers of organic origin (Commission Regulation, 2008). However, this jeopardizes the credibility of organic-certified products due to the uncertain origin of these fertilizers. In Denmark, organic farmer associations have formulated a joint appeal to stop the use of animal-based fertilizers from conventional farming, aligned with the expected development of new alternative fertilizers of organic origin. Currently, organic growers can apply up to 50 kg plant-utilized N ha⁻¹ year⁻¹ of conventional animal-based fertilizer (Landbrugsstyrelsen, 2021a). The main part of this fertilizer used in Denmark is cattle and pig slurry. Nutrients in slurry are easily available for plants, as they are primarily dissolved in mineral form.

The lack of fertilizers of organic origin is especially pronounced in the production of organic vegetable crops that often have high nutrient demands and are grown in areas with limited production of animal-based manure (Landbrugsstyrelsen, 2021b). Therefore, green manures are commonly used in organic vegetable production to improve soil fertility and fertilization of N demanding crops. However, the traditional legume-based green manures are incorporated before the next crop is planted or sown and do not allow split-dose fertilizer application during the growing season. This can lead to poor timing between nutrient release and nutrient demand by crops (Xie et al., 2018). An alternative practice to optimize the fertilizer value of green manure is to use it as a fertilizer in nearby fields during the growing season (Sorensen & Thorup-Kristensen, 2011; Van der Burgt et al., 2013). Repeated cutting of green manure crops during the growing season increases cumulative biomass (Dahlin & Stenberg, 2010; Råberg et al., 2018). The decomposition of this biomass is a critical step in releasing nutrients by net-mineralization. Beside soil humidity and temperature, decomposition and net-mineralization are strongly affected by the C:N ratio of the biomass (Frankenberger & Abdelmagid, 1985; Marstorp & Kirchmann, 1991). A maximum of 15 was previously estimated to avoid immobilizing nutrients during decomposition (Marstorp & Kirchmann, 1991). Furthermore, the rate of decomposition is influenced by whether green manure is incorporated into the soil or is left on the soil surface (Coppens et al., 2007; Poffenbarger et al., 2015).

The objective of this study was to (1) maximize the annual N production of green manure crops, (2) optimize the C:N ratio of green manure crops, (3) quantify the fertilizer value of green manure when applied to vegetable crops, and (4) compare soil incorporation to surface application.

We hypothesized that more than 500 kg N ha⁻¹ year⁻¹ of plant-based manure with low C:N ratios could be produced, with fertilizer values comparable to those of animal-based manure, providing an alternative to fertilizers from conventional farming in organic vegetable production. To test this hypothesis, we measured the cumulative N production and C:N ratio of perennial and annual plant species

(mainly legumes) cut several times at different developmental stages. We also tested the effect of plant-based fertilization on biomass yield, N offtake, and N recovery of white cabbage (*Brassica oleracea* L. var. *capitata* L.) and leek (*Allium porrum* L.) in comparison to fertilization by poultry manure and an unfertilized treatment.

2 | MATERIALS AND METHODS

2.1 | Site, soil, and climate

The experiments were conducted at Aarhus University, Aarslev, Denmark (55°18'N, 10°27'E). The climate is temperate coastal, with monthly mean temperatures of 10–17°C from May to October (1991–2020). The experiments were carried out in an organically managed farming system on sandy loam soil (Typic Agrudalf) during 2012–2016. The texture of the top soil consisted of 12% clay, 15% silt, 70% sand, and 2.8% soil organic matter. During April of each year, the pH and content of plant-available N, phosphorus (P), and potassium (K) in the top soil were recorded (Table 1).

2.2 | Production of plant-based fertilizers

The production potential of perennial and annual green manure crops was examined by harvesting plots of 11.7 m². At each cut of all green manure crops, stubble height was 9 cm. Fresh matter was weighed and plant samples were dried and analyzed for total N and C content. The cumulative N production equals the cumulative N offtake by green manure crops. All experiments were arranged in randomized complete block designs with three replicates. No fertilizer was applied to green manure crops.

2.2.1 | Perennial green manure crops

Alfalfa (*Medicago sativa* L. “Creno”), white clover (*Trifolium repens* L. “Klondike”), red clover (*Trifolium pratense* L. “Rajah”), and a grass-clover mixture (70% Italian ryegrass [*Lolium multiflorum* Lam. “Foxtrot”] and 30% red clover “Rajah”) were undersown in spring barley (*Hordeum vulgare* L.) in April of 2012 and 2013. Seed density was 30, 10, 15, and 30 kg ha⁻¹, respectively. The following year (2013 and 2014), perennial crops were cut at three developmental stages: early, intermediate, and late. The intermediate stage corresponded to the initial flowering stage, with early and late stages being approximately 2 weeks earlier and 2 weeks later, respectively. The first cuts at the early stage were implemented on May 24, 2013 and May 14, 2014. The crops were cut two to five times at the same three developmental stages, that is, the early-stage crops required approximately 5–7 weeks to regrow and reach the early stage for the next cutting, while intermediate

TABLE 1 Plant-available N, P, and K and pH in the 0–0.25 m soil layer in April 2012–2014 with experiments on plant-based fertilizer production and in 2015 and 2016 with experiments on fertilizer value of plant-based fertilizers

	Experiments on N production by plant-based fertilizer			Experiments on N fertilizer value of plant-based fertilizer		
	2012	2013	2014	White cabbage	Leek	2016
N _{min} (kg ha ⁻¹)	62	22	22	40	35	37
P (mg kg ⁻¹)	27	45	21	26	21	21
K (mg kg ⁻¹)	170	190	166	130	120	93
pH	6.2	6.4	6.2	5.7	5.8	5.8

stage crops required 7–9 weeks to regrow between cuttings, and late-stage crops required 9–11 weeks. In 2014, green manure crops were irrigated twice with 25 L m⁻² to mitigate severe drought stress.

2.2.2 | Annual green manure crops

Broad bean (*Vicia faba* L. “Colombo”), blue lupine (*Lupinus angustifolius* L. “Viol”), and pea (*Pisum sativum* L. “Alvesta”) were sown in April of 2012 and 2013. Seed density was 450, 200, and 200 kg ha⁻¹, respectively. Lupine was inoculated with *Bradyrhizobium japonicum*. These annual crops were cut at three developmental stages (early, intermediate, and late) with 1-week intervals. Plant age at the early stage was 7–8, 8–9, and 9–10 weeks for pea, lupine, and broad bean, respectively. The first cuts at the early stage were implemented on June 18, 2012 and June 25, 2013. These annual crops were harvested for seeds at maturity as a fourth stage of development. Annual crops were resown in the same plots in the same year after the first cut, except in 2013 when red clover was undersown just after sowing lupine. The previous year, the crop was spring barley followed by bare soil over winter.

2.3 | Fertilizer value of plant-based fertilizers

The fertilizer value of the green manure was tested by measuring the harvested biomass, N offtake, and N recovery of field grown white cabbage (*Brassica oleracea* L. var. *capitata* L.) in 2015 and leek (*Allium porrum* L.) in 2015 and 2016. N offtake was determined as the multiple of aboveground biomass and the concentration of N in the biomass. Apparent fertilizer-N recovery was determined as the difference in N offtake in vegetable plant biomass between fertilized and unfertilized treatments divided by the amount of applied total N.

2.3.1 | White cabbage experiment

In 2015, the fertilizer value of alfalfa and clover (60% red clover and 40% white clover) was tested on white cabbage (cv. Kilazol). Control treatments included unfertilized plots and plots fertilized with pellets of dried poultry manure (Farmergødning 3-1-2, Farmergødning I/S, Aalestrup, Denmark). The green manure was produced in 2014 from cuttings at both early and late developmental stages. Subsequently, the

green manure crops were dried and pelleted at Farmergødning I/S. The average C:N ratio of alfalfa and clover pellets produced from plants cut at the early and late developmental stages was 12.0 and 17.6, respectively. The C:N ratio of poultry manure pellets was 10.8. N_{min} in the top 0.5 m soil layer was 69 kg N ha⁻¹ on June 8, 2015. Two thirds of the pellets of green manure crops and poultry manure were incorporated into the top 0.1 m soil layer on June 11, and after rotovating the soil, cabbage plants were transplanted. Four weeks later, the final part of the green manure and poultry manure was placed between plant rows and incorporated by a row rotovator. In total, 220 kg N ha⁻¹ was applied for all treatments. The experiment was arranged in a randomized complete block design with three replicates. Plot size was 16 m², plant density was 3.0 plants m⁻², and the plant biomass (cabbage heads and outer leaves) from 4.3 m² was determined on October 16.

2.3.2 | Leek experiment

In 2015 and 2016, fresh green manure crops were used directly as fertilizer for leek. Control treatments included unfertilized plots and plots fertilized with dried poultry manure (Binadan 4-1-3, Binadan A/S, Denmark). The initial amount of N_{min} in the top 0.5 m soil layer was 60 and 58 kg N ha⁻¹ on June 8, 2015 and May 18, 2016, respectively. Leek plants were transplanted on June 18, 2015 and June 3, 2016.

In 2015, the green manure was alfalfa, white clover, and red clover cut at an early stage of development. Sixty percent of the green manure was incorporated in the top 0.1 m soil layer by soil rotovation 1 week before transplanting leek (cv. Catcher). Five weeks later, the final parts of the green manure crops were placed between plant rows and incorporated into the soil by a row rotovator. The intended amount of total N applied with fresh green manure crops and poultry manure was 225 kg N ha⁻¹. However, N concentration could not be determined before application, and applied N turned out to be 236, 191, 243, and 204 kg N ha⁻¹ for alfalfa, white clover, red clover, and poultry manure, respectively. The average C:N ratio of alfalfa, white clover, red clover, and poultry manure was 13.2, 11.7, 13.9, and 8.8, respectively.

In 2016, the green manure crops consisted of clover (60% red clover and 40% white clover), which was cut every third, fourth, and sixth week. Twenty percent of the green manure and poultry manure were applied on May 20, 2016, 2 weeks before transplanting leek (cv. Belton). The last 80% were split into four, three, and two applications for the 3-, 4-, and 6-week-old cuttings, respectively, and three applications

for poultry manure. The final application was carried out 75 days after transplanting. Green manures and poultry manures were placed on the soil surface between plant rows with and without soil incorporation by a row rotovator into the top 0.1 m soil layer. The intended amount of total N applied was 225 kg N ha⁻¹; however, plant analysis showed that 228–234 kg N ha⁻¹ were actually applied. The average C:N ratio of green manure cut every third, fourth, and sixth week was 12.5, 13.5, and 15.7, respectively, and that of poultry manure was 9.4.

In both years, the experiment was arranged in a randomized complete block design with four replicates. Plot size was 16 m², plant density was 18.8 plants m⁻², and plant biomass (whole plants without roots) was determined on 4.3 m² on October 20, 2015 and October 17, 2016.

2.4 | Sampling and analysis

During the growth of white cabbage and leek, the top 0.25 m soil layer was sampled repeatedly using a hand-driven auger. Soil samples were stored at -18°C until analysis for N_{min}, which was determined by immediate extraction of 100 g thawed soil with 0.2 L of 1.0 M KCl for 1 h. The extract was filtered and analyzed for nitrate and ammonium content using standard colorimetric methods (Sørensen & Bülow-Olsen, 1994) in an AutoAnalyzer 3 (Bran+Luebbe GmbH, Norderstedt, Germany).

The plant-available pool of soil P was determined by extracting it from 5 g soil with 0.5 M NaHCO₃, adjusted to pH 8.5 for 0.5 h at 20°C. Immediately after, the soil and solution were separated. In a clear filtrate, the concentration of the blue phosphomolybdate complex was measured by spectrophotometry after adding sulfuric acid, ascorbic acid, and ammonium molybdate reagent to the extract (Rubæk & Kristensen, 2017). Exchangeable K was measured by flame photometry after extracting it from 10 g soil with 100-mL reagent (0.5 M CH₃COONH₄ and 3 mM LiCl) (Sørensen & Bülow-Olsen, 1994). pH was determined by an ion selective glass electrode after suspending 10 g soil in 25-mL 0.01 M CaCl₂ solution (Sørensen & Bülow-Olsen, 1994).

The green manure was analyzed for total N and C content, and the vegetable samples (six cabbage plants or 20 leek plants) were analyzed for total N. Plant samples were chopped and dried at 80°C to a constant weight. Total plant N was analyzed according to Anonymous and VDLUFA (1991), in which plant material was burned at 950°C and the N concentration was determined as molecular N using a LECO TruSpec CN (LECO Corporation, St. Joseph, Michigan, USA). Total organic C concentration was determined by the dry combustion method of Dumas, in which plant material is burned at 1000°C and total organic carbon concentration is measured by an ELTRA Helios CS-analyzer (Eltra GmbH, Haan, Germany).

2.5 | Data and statistical analysis

Results were calculated as an average of replicates for each treatment. Analysis of variance was performed on each variable using the Statistical Analysis System (SAS Institute Inc., 1989). The effects were tested

using the General Linear Models procedure. Results were considered significant when $p \leq 0.05$, according to this procedure. For each variable, treatment means were separated by the Tukey test at the 5% level. The CORR procedure was used to test correlations between variables.

3 | RESULTS

3.1 | Production strategy and quality of plant-based fertilizer

The N production at the first cut of green manure crops increased with plant age (Figures 1 and 2). For perennial crops, the number of cuts during one season decreased when the period of regrowth increased. At early, intermediate, and late growth stages, the number of cuts was four, three, and two, respectively. In 2014, the number of cuts of alfalfa was higher, and increased to five, four, and three at the three developmental stages. In 2013, cumulative N production over the entire growing season increased with more frequent cuts, except for alfalfa. In 2014, however, cumulative N production was not significantly influenced by the cutting frequency. In 2013, the maximum N production was 520 kg N ha⁻¹ for alfalfa and red clover, and 390 kg N ha⁻¹ for white clover. In 2014, N production further increased by 60 kg ha⁻¹, on average, especially in alfalfa, due to the increased number of cuts. Maximum cumulative N production was 640 kg ha⁻¹ for alfalfa at the late stage.

For annual crops, N production at the first cut increased with plant age (Figure 2). By re-sowing, the cumulative N production in broad bean and lupine was 200–300 kg ha⁻¹. At the intermediate stage of broad bean and lupine, re-sowing failed in 2012. In 2013, the second crop of undersown red clover in lupine produced 150 kg N ha⁻¹, in addition to the N production of lupine at the first cut. When only harvesting the seeds of broad bean and lupine, N production was around 200 kg ha⁻¹. The N production of pea seeds in 2013 was 152 kg ha⁻¹.

The C:N ratio of perennial green manure crops increased with developmental stage (Table 2). At the first cut in May, the average C:N ratio increased from 11 at the early stage to 14 and 17 at the intermediate and late stages, respectively. At later cuts during the season, this increase was less clear; however, the C:N ratio increased from 13 at the early stage to 15 and 17 at the intermediate and late stages, respectively. The average C:N ratio of white clover, alfalfa, red clover, and grass clover was 12.8, 13.4, 14.4, and 14.9, respectively.

For the annual crops, the C:N ratio increased from 13.3 to 14.3 from the early to late stage (average of species, dates, and years) (Table 3). The C:N ratio of broad bean, lupine, and pea was 12.2, 13.9, and 15.6, respectively. The lowest C:N ratio (8.7) was obtained for the seeds of broad bean and lupine, whereas that of pea seeds was 14.0.

3.2 | Fertilizer value of plant-based fertilizers

Application of plant-based fertilizer for white cabbage and leek increased plant biomass and N offtake. However, in general the fertilizer value of plant-based fertilizer was not different from poultry manure.

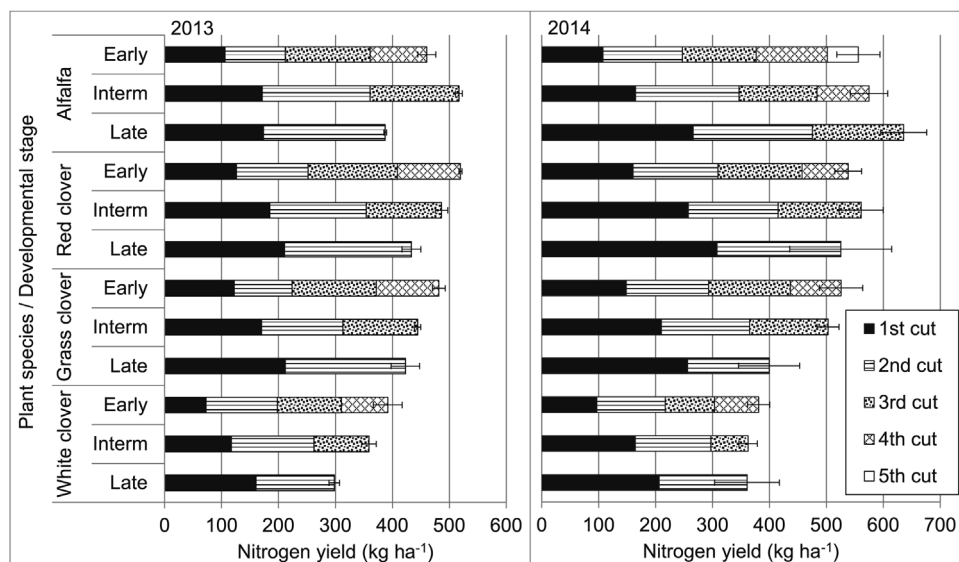


FIGURE 1 Cumulative production of aboveground N for perennial green manure crops cut and re-cut at three stages of plant development over 2 years. Bars indicate \pm standard errors on the means ($n = 3$).

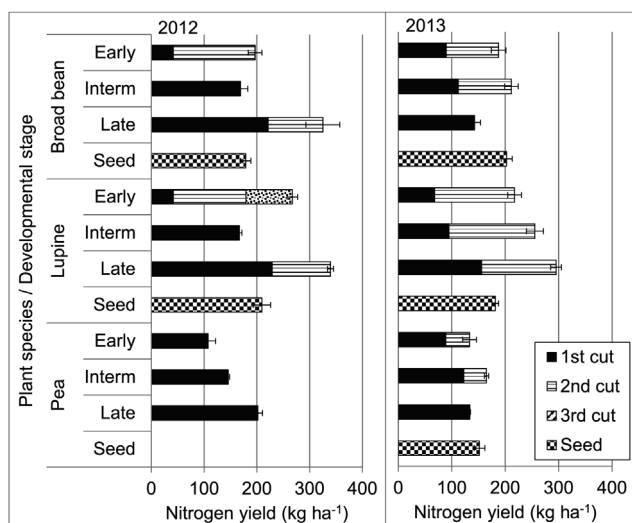


FIGURE 2 Cumulative production of aboveground N for annual green manure crops cut and re-cut at four stages of plant development over 2 years. The second harvest of lupine in 2013 was undersown red clover. Bars indicate \pm standard errors on the means ($n = 3$).

In 2015, pelleted alfalfa and clover mixture applied as the sole fertilizer for white cabbage resulted in a 6%–8% increase in plant biomass and 12%–29% increase in N offtake compared to poultry manure, when cut at a young stage (C:N ratio: 12) (Table 4). The fertilizer value was independent of cutting stage of both alfalfa and clover, although this result was less clear for clover.

Application of fresh plant-based fertilizer to leeks in 2015 resulted in comparable plant biomass compared to poultry manure (Table 5). The N offtake was also comparable except for red clover that reduced the N offtake by 18% compared to poultry manure. The amount of N applied with alfalfa and red clover was slightly higher (5% and

8%, respectively) compared to the intended application of 225 kg N ha⁻¹. The low N offtake of leek with the slightly higher N application of red clover resulted in a low apparent N recovery. In contrast, the amount of N applied with white clover was 15% lower compared to the intended N application, resulting in high apparent N recovery.

In 2016, split application of a soil incorporated clover mixture cut at a very early stage (i.e., cut every 3 or 4 weeks) resulted in plant biomass equal to that of poultry manure (Table 6). However, incorporated clover mixture cut every 3 or 6 weeks reduced N offtake and apparent N recovery compared to poultry manure.

Surface application of plant-based fertilizers reduced biomass and N offtake of leek by 10% ($p \leq 0.01$) and 26% ($p \leq 0.001$), respectively, resulting in a 52% ($p \leq 0.001$) lower N recovery compared to soil incorporation of the fertilizer crops.

Soil incorporation of the applied fertilizer increased N_{\min} in the top soil. Four weeks after applying 150 kg N ha⁻¹, soil N_{\min} increased by approximately 120 kg ha⁻¹ when the C:N ratio of the plant-based fertilizers was 12 (Figure 3). However, the C:N ratio of plant-based fertilizers applied to both white cabbage and leek was not correlated to N_{\min} measured 3–4 weeks after fertilizer application. With white cabbage, soil N_{\min} decreased rapidly, whereas a more gradual decline was observed with leeks. Three weeks after applying 48 kg N ha⁻¹, with either green manure or poultry manure in May 2016, N_{\min} increased by 20 and 30 kg ha⁻¹ in the top soil, respectively. In general, incorporation of poultry manure resulted in higher soil N_{\min} , during the growing period, compared to the incorporation of clover mixture. With surface application, soil N_{\min} remained low, and was not more than 20 kg ha⁻¹ higher compared to unfertilized soil.

The N offtake by white cabbage and leek was both positively correlated to N_{\min} measured 3–4 weeks after fertilizer application ($p \leq 0.05$; $R^2 = 0.83$ – 0.94).

TABLE 2 Carbon-to-nitrogen (C:N) ratio of the aboveground parts of perennial green manure crops cut and re-cut at early, intermediate, or late stage of plant development in 2013 and 2014. Means ($n = 3$) within each cut followed by different letters are statistically different among treatments (ns = not significant, * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$; Tukey test)

Green manure	Stage	Cut in 2013				Cut in 2014				
		1 st	2 nd	3 rd	4 th	1 st	2 nd	3 rd	4 th	5 th
Alfalfa	Early	10.1 ^{de}	10.6 ^e	14.3 ^{ab}	12.5 ^a	11.8 ^d	13.4 ^d	13.2 ^c	11.1 ^b	11.2
	Intermediate	13.3 ^{cd}	16.0 ^{abc}	13.9 ^{bc}		13.1 ^{cd}	15.8 ^{abcd}	13.0 ^c	13.0 ^a	
	Late	17.3 ^{ab}	17.1 ^{ab}			14.8 ^{abcd}	15.0 ^{abcd}	14.3 ^{abc}		
Red clover	Early	10.2 ^{de}	12.5 ^{de}	16.1 ^a	13.1 ^a	12.7 ^{cd}	15.5 ^{abcd}	15.4 ^a	12.5 ^{ab}	
	Intermediate	14.0 ^{bc}	16.8 ^{abc}	15.0 ^{ab}		13.8 ^{bcd}	16.5 ^{ab}	13.1 ^c		
	Late	19.2 ^a	16.5 ^{abc}			17.3 ^{ab}	15.1 ^{abcd}			
Grass clover	Early	11.4 ^{cde}	14.1 ^{cd}	15.9 ^a	13.4 ^a	14.3 ^{bcd}	17.1 ^a	15.2 ^{ab}	11.8 ^{ab}	
	Intermediate	14.3 ^{bc}	18.9 ^a	15.3 ^{ab}		15.6 ^{abc}	15.9 ^{abc}	13.7 ^{bc}		
	Late	18.3 ^a	17.7 ^{ab}			18.1 ^a	16.1 ^{abc}			
White clover	Early	9.5 ^e	12.2 ^{de}	15.3 ^{ab}	11.2 ^b	12.2 ^{cd}	14.4 ^{bcd}	13.8 ^{bc}	11.1 ^b	
	Intermediate	12.5 ^{cde}	18.4 ^a	12.0 ^c		12.2 ^{cd}	13.9 ^{cd}	10.7 ^d		
	Late	14.2 ^{bc}	14.9 ^{bcd}			15.4 ^{abcd}	14.0 ^{bcd}			
Significance _{manure}		***	***	***	**	***	***	***	ns	–
Significance _{stage}		***	***	***	–	***	ns	***	**	–
Significance _{manure × stage}		ns	*	*	–	ns	*	**	–	–

4 | DISCUSSION

4.1 | Production strategy and quality of plant-based fertilizers

4.1.1 | Effect of species and number of cuts on cumulative N production

Alfalfa, red clover, and grass clover produced around 500 kg N ha⁻¹, with maximum production of 640 kg N ha⁻¹ (Figure 1). Previous studies obtained lower or almost as high cumulative productions of 230–470 kg N ha⁻¹ when re-cutting grass clover leys or green manure in the Netherlands (Elgersma et al., 1998; Giambalvo et al., 2011; Råberg et al., 2018; Stopes et al., 1996; Vinther, 2006). Råberg et al. (2018) reported a production of 470 kg N ha⁻¹ in southern Sweden for perennial green manure ley composed of alfalfa, red clover, yellow sweet clover (*Melilotus officinalis* L.), orchard grass (*Dactylis glomerata* L.), meadow fescue (*Festuca pratensis* L.), and timothy grass (*Phleum pratense* L.). This N production was obtained by three cuts in 1 year. However, N production in the previous year was about half (230 kg N ha⁻¹), demonstrating variable cumulative N production depending on growing conditions, supported by our study.

The highest cumulative N productions obtained in 2014 exceeded the productions documented in the published literature. These productions were attributed to a combination of higher temperature compared to 2013 (higher daily temperature of 1.1°C on average from April to October), and well-timed and conducted management in rela-

tion to weather conditions. In particular, the crops were irrigated in 2014 to mitigate severe drought stress.

Maximizing the number of cuts of perennial green manure crops increased cumulative N production. Although N production increased with developmental stage at the first cut, re-cutting during early and intermediate developmental stages with three to four cuts generated the highest cumulative N production. For alfalfa in 2014, the latest developmental stage produced the highest production. Trials with alfalfa showed that, when an extra cut for each developmental stage (three to five cuts instead of two to four cuts) was applied in 2014, the N production was higher (by 80 kg N ha⁻¹) compared to red clover and clover mixture. In contrast, in 2013, crops had the same number of cuts and N production. This result supports that maximizing the number of cuts is beneficial. Egyptian clover (*Trifolium alexandrinum* L.) and a mixture of Egyptian clover and ryegrass increased cumulative N production when re-cut four to five times instead of two (Giambalvo et al., 2011). However, the cutting frequency has an upper limit, as demonstrated by Vinther (2006), whereby seven cuts led to a 30 kg N ha⁻¹ reduction in N production compared to three cuts.

The cumulative N production of white clover in the current study was low compared to the other perennial legumes, which was attributed to a lower biomass (data not shown). Similarly, a low cumulative N production of 290 kg N ha⁻¹ was found for a mixture of white clover and perennial ryegrass (*Lolium perenne* L.) (Vinther, 2006). The N production of white clover was lower compared to red clover, even when biomass production was higher (Stopes et al., 1996). The N production of white clover is, however, influenced by the cultivar (Elgersma & Hassink, 1997).

TABLE 3 Carbon-to-nitrogen (C:N) ratio of the above-ground parts of annual green manure crops cut and re-cut at early, intermediate, late, and seed stages of plant development in 2012 and 2013. Means ($n = 3$) within each cut followed by different letters are statistically different among treatments (ns = not significant, * $p \leq 0.05$, and *** $p \leq 0.001$; Tukey test)

Green manure	Stage	Cut in 2012		Cut in 2013	
		1 st	2 nd	1 st	2 nd
Broad bean	Early	11.6 ^{bc}	10.9 ^b	12.2 ^{cd}	11.2 ^b
	Intermediate	11.3 ^c		14.0 ^{bc}	11.8 ^b
	Late	12.9 ^{abc}	12.0 ^{ab}	16.1 ^{bc}	
	Seed	8.1 ^d		9.0 ^d	
Lupine	Early	14.7 ^a	14.0 ^a	13.1 ^c	15.5 ^a
	Intermediate	12.6 ^{bc}		14.3 ^{bc}	14.8 ^a
	Late	14.8 ^a	11.6 ^{ab}	14.2 ^{bc}	14.5 ^a
	Seed	8.5 ^d		9.0 ^d	
Pea	Early	11.7 ^{bc}		14.9 ^{bc}	16.7 ^a
	Intermediate	12.1 ^{bc}		17.3 ^{ab}	16.6 ^a
	Late	13.5 ^{ab}		20.6 ^a	
	Seed			14.0 ^{bc}	
Significance _{manure}		***	*	***	***
Significance _{stage}		***	ns	***	ns
Significance _{manure × stage}		*	*	ns	ns

TABLE 4 Fertilizer value (dry matter biomass, N offtake, and apparent fertilizer-N recovery) of dried green manure and poultry manure for white cabbage grown in 2015. The green manure consisted of alfalfa, white clover, and red clover cut at early and late developmental stage. Means ($n = 3$) followed by different letters are statistically different among treatments (ns = not significant and *** $p \leq 0.001$; Tukey test)

Manure	Stage	C:N	Biomass (Mg ha ⁻¹)	N offtake (kg ha ⁻¹)	N recovery (%)
Alfalfa	Early	12.0	10.5 ^{ab}	200 ^a	49 ^a
Clover	Early	12.1	10.3 ^{ab}	174 ^a	37 ^a
Alfalfa	Late	17.5	10.8 ^a	198 ^a	48 ^a
Clover	Late	17.6	8.9 ^b	144 ^{ab}	23 ^a
Poultry manure		10.8	9.7 ^{ab}	155 ^a	29 ^a
Unfertilized			6.5 ^c	92 ^b	
Significance			***	***	ns

TABLE 5 Fertilizer value (dry matter biomass, N offtake, and apparent fertilizer-N recovery) of fresh green manure and poultry manure for leek grown in 2015. The green manure consisted of alfalfa, white clover, and red clover cut at an early stage. Means ($n = 4$) followed by different letters are statistically different among treatments (** $p \leq 0.001$; Tukey test)

Manure	N applied (kg ha ⁻¹)	C:N	Biomass (Mg ha ⁻¹)	N offtake (kg ha ⁻¹)	N recovery (%)
Alfalfa	236	13.2	6.1 ^a	125 ^a	33 ^b
White clover	191	11.7	5.7 ^a	127 ^y	43 ^a
Red clover	243	13.9	5.4 ^a	97 ^y	20 ^c
Poultry manure	204	8.8	5.5 ^a	114 ^a	33 ^b
Unfertilized	0		4.0 ^b	47 ^c	
Significance			***	***	***

TABLE 6 Fertilizer value (dry matter biomass, N offtake, and apparent fertilizer-N recovery) of fresh clover mixture (60% red clover and 40% white clover) and poultry manure for leek grown in 2016. The fertilizer was either surface applied or soil incorporated. The clover mixture was cut every third, fourth, and sixth week, resulting in five, four, and three applications, respectively. Means ($n = 4$) followed by different letters are statistically different among treatments (** $p \leq 0.01$ and *** $p \leq 0.001$; Tukey test)

Manure	N applied kg ha ⁻¹	C:N	Biomass Mg ha ⁻¹	N offtake kg ha ⁻¹	N recovery %
Surface applied					
3-week clover	228	12.5	8.0 ^{ab}	108 ^c	17 ^c
4-week clover	231	13.5	8.1 ^{ab}	110 ^{bc}	18 ^{bc}
6-week clover	234	15.7	7.3 ^{ab}	93 ^{cd}	10 ^c
Soil incorporated					
3-week clover	228	12.5	8.9 ^a	136 ^b	29 ^b
4-week clover	231	13.5	9.1 ^a	164 ^a	42 ^a
6-week clover	234	15.7	7.9 ^{ab}	121 ^{bc}	22 ^{bc}
Poultry manure	231	9.4	8.0 ^{ab}	166 ^a	42 ^a
Nonfertilized	0		6.3 ^b	70 ^d	
Significance			**	***	***

The cumulative N production of annual green manure crops was 44% lower compared to that of perennial green manure crops, as annual species cannot be re-cut. This distinct difference among the crop species seems to be clearly essential for maximizing N production. The re-sowing of annual crops led to a longer interval between cuts compared to re-cutting perennials, which are able to regrow from the stem base connected to a well-established root system. In the current study, wet and dry conditions at re-sowing hindered seedbed preparation and enhanced weed problems and soil-borne diseases. Therefore, only one to three cuts were possible for annual crops compared to two to five cuts for perennial crops.

To our knowledge, the N productions of annual legume crops obtained by re-sowing and cutting have not been previously reported. The highest productions in the current study of 335 kg N ha⁻¹ for broad bean and lupine in 2012 were obtained when cutting at the late developmental stage, and when re-sowing and two cuts were possible. In peas, re-sowing caused N production to drop, due to poor plant emergence and poor plant growth caused by blight (*Ascochyta* spp.). Our study obtained similar N productions to previous studies when harvesting the whole crop at seed maturity. For instance, 340 kg N ha⁻¹ was obtained for broad bean (Kaul et al., 1996) and up to 300 kg N ha⁻¹ for pea (Hauggaard-Nielsen & Jensen, 2001).

4.1.2 | C:N ratio of plant-based fertilizers

The C:N ratio for the first cut of the perennial green manure crops increased from an average of 11.5 at the early developmental stage to 16.8 at the late stage. For annual crops, the C:N ratio increased from 13.1 at the early developmental stage to 15.4 at the late stage. This change in elemental concentration supports existing studies (Rominger et al., 1975; Sørensen, 1992), and is caused by a decrease in the

leaf:stem ratio, which increases the concentration of carbon-rich constituents (Jarrell & Beverly, 1981). At the second and later cuts, the C:N ratio also increased with plant age, but less clear. For the perennial crops, interactive effects were seen at the second and third cut in both years indicating a poor relationship between the developmental stage and the C:N ratio. At the early stage, the C:N ratio was high at the second and third cut of some crops compared to the first cut indicating that the regrowth period of 5–7 weeks could be shorter. For the annual crops, interactive effects were seen in 2012 where the C:N ratio of lupine and broad bean at the intermediate stage was low compared to the early and late stages, indicating that the first cut at the early stage should be even earlier. A low C:N ratio of 8.7 was obtained for the seeds of broad bean and lupine, which was similar to that obtained for poultry manure, and supported previous findings (Li et al., 2015). Green manure cut at the late developmental stages had C:N ratios slightly higher than 15, which is the point at which net mineralization switches to net immobilization in incorporated green manure (Marstorp & Kirchmann, 1991).

To conclude, our findings support the hypothesis that more than 500 kg N ha⁻¹ year⁻¹ of green manure crops with low C:N ratios could be produced.

4.2 | Fertilizer value of plant-based fertilizers

4.2.1 | Biomass of vegetable crops and soil mineral nitrogen

The biomass of white cabbage and leek fertilized with incorporated green manure crops was comparable to poultry manure. This finding supports a previous study showing comparable or higher yields of spinach (*Spinacia oleracea* L.) when fertilized with fresh alfalfa and

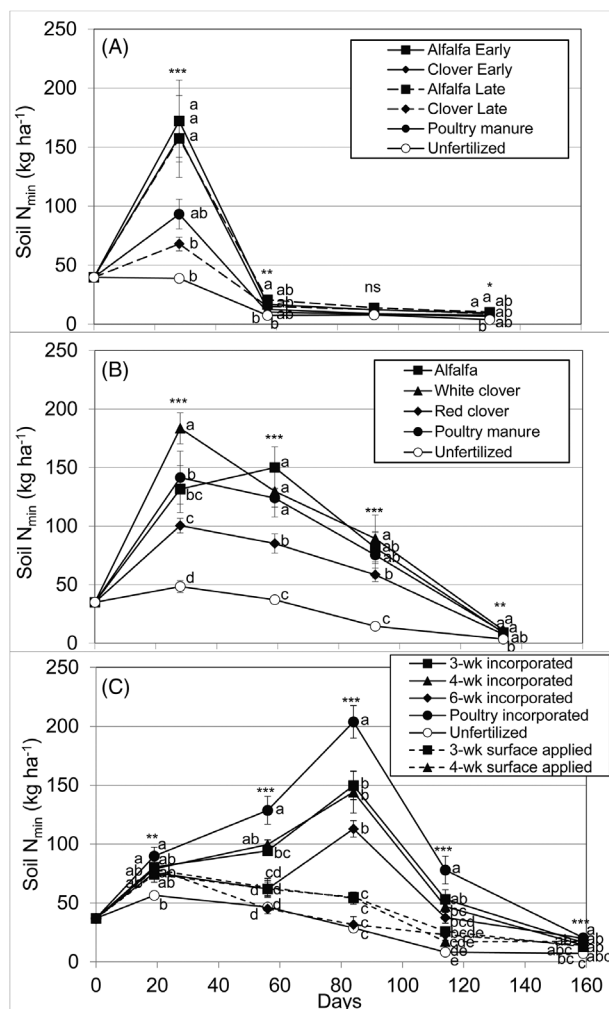


FIGURE 3 Relationship between days after first fertilizer application and soil mineral N on the top 0.25 m soil profile with: (A) incorporated alfalfa and mixed clover from early and late stage cuts applied to white cabbage in 2015, (B) incorporated alfalfa, white clover, and red clover from early stage cuts applied to leek in 2015, and (C) mixed clover cuts every third, fourth, and sixth week (five, four, and three applications, respectively), incorporated in the topsoil or left on the soil surface of leek crops in 2016. Bars indicate \pm standard errors on the means of three (A) or four (B and C) replicates and different letters indicate statistical difference within each day of sampling and experiment ($p \leq 0.05$; Tukey test).

grass clover compared to poultry manure (Van der Burgt et al., 2013). Toleikiene et al. (2020) also showed that fresh red clover caused wheat (*Triticum aestivum* L.) grain yield to increase by 10% compared to granulated cattle manure on a loam soil, but not a clay loam soil.

Plant-based fertilizers had a favorable effect on cabbage and leek biomass, despite having a higher C:N ratio compared to poultry manure (C:N ratios: 11.7–17.6 and 8.8–10.8, respectively). This finding could be explained by the 20%–40% higher application of organic matter with plant-based fertilizers having a positive effect on crops. Organic matter is important to several characteristics of soil fertility, including water holding capacity and microbial activity.

However, these findings contrast with the claim that low C:N ratios are required for plant-based fertilizer with high short-term N availability, and the fact that a C:N ratio below 15 is considered to be the turning point to net mineralization (Marstorp & Kirchmann, 1991). However, mineralization is strongly influenced by the chemical compounds in organic matter, as well as temperature and moisture content in soil, and is considered difficult to predict (Andersen & Jensen, 2001; De Neve, 2017). Despite this, a significant correlation between vegetable biomass and the C:N ratio of green manure was demonstrated in 2 out of 3 years by Sorensen and Thorup-Kristensen (2011).

The soil N_{min} measured after applying organic fertilizer was expected to be correlated to C:N ratio. However, application of alfalfa, cut at the late stage of development in 2015, resulted in similar N_{min} levels as early-stage cut alfalfa and clover mixture. In contrast, late-stage cut clover acted as expected, with N_{min} levels below that of poultry manure. In addition, application of alfalfa resulted in higher N_{min} compared to red clover without increase in leek biomass in 2015. The nutrient status of plant material did not seem to affect mineralization, because P, K, and S content in the present study was comparable among alfalfa, clover mixture, and red clover (data not shown). This finding might be attributed to differences in the application of organic matter among the green manure crops, or species-specific differences in the content of organic substances, such as phenols and plant hormones not identified in this study. Bruulsema and Christie (1987) measured a higher fertilizer value for alfalfa compared to red clover in a subsequent corn (*Zea mays* L.) crop. Moreover, they indicated that differences in decomposability were caused by differences in the C:N ratio and lignin content. Lignin content is known to be higher in alfalfa compared to red clover (Marković et al., 2010).

Soil N_{min} was higher when the green manure crops were incorporated compared to surface application of the same type and amount of fertilizers; yet, leek biomass was just 10% lower after surface application. Part of the N applied in the green manure crops might have been lost by ammonia volatilization, and the N released from mineralization might have been reduced and delayed. Despite this, N from surface applied green manure crops was sufficient to support leek growth.

4.2.2 | N offtake and recovery by vegetable crops

Positive correlations were obtained between N offtake by vegetable crops and N_{min} levels in soil after incorporating green manure, whereas the amount of N applied showed contradictory results. For instance, the 190 kg N ha⁻¹ applied with white clover to leek in 2015 led to significantly higher N offtake compared to the 204 kg N ha⁻¹ applied with poultry manure. Furthermore, the 243 kg N ha⁻¹ applied with red clover led to a significantly lower N offtake. Thus, the relationship between total N applied and vegetable N accumulated is species specific for the plant-based fertilizers, supporting the findings of Sorensen and Thorup-Kristensen (2011), who ascribed the correlation between N applied and N accumulated in vegetables to

species-specific differences in the rate of N release from green manure species.

N offtake in white cabbage and leek was higher (by approximately 35 kg N ha⁻¹) when the green manure was soil incorporated compared to surface application. This result was attributed to higher N_{min} being caused by faster N release, supporting the work of Coppens et al. (2007) and Radicetti et al. (2016).

While significant differences in N recovery between plant-based fertilizers and poultry manure in white cabbage and leek were obtained, it differed which green manure crop led to the highest recovery. In a spinach crop, plant-based fertilization by alfalfa and grass clover led to higher apparent N recovery compared to poultry manure (Van der Burgt et al., 2013), whereas cattle slurry was superior to plant-based fertilization for spring barley (Sørensen et al., 2013).

In conclusion, this study on white cabbage and leek supports our hypothesis that the fertilizer value of plant-based fertilizer is comparable to animal-based manure on the condition that the C:N ratio is <18 and that the fertilizer is soil incorporated.

4.3 | Perspectives for organic crop production

The credibility of the organic-certified plant food products increases when green manure is harvested and used as fertilizer in nearby fields as a substitute for animal-based fertilizer from conventional farming. However, the production of plant-based fertilizers utilizes land for the production of sales crops. By comparing three organic crop rotations with different areas of traditional grass-clover green manure, Pedersen et al. (2014) showed that the highest economic return could be achieved when 20% of land was used to produce plant-based fertilizers in a 10-year crop rotation with cereals and pulses. The economic return increases further when perennial green manure crops are cut several times each year compared to traditional green manure, and when vegetables or other high-value crops are produced compared to agricultural crops.

Although green manure grown in a production field increases expenses to land and cutting, this type of fertilizer is considered competitive to other fertilizers used in the production of organic high-value crops, because other fertilizers also incur the expense of transportation and application. However, our study showed that the production of plant-based fertilizers must be based on perennial legumes or grass clover, which can be cut repeatedly each year, as annual legumes had lower N productions. Furthermore, the re-sowing strategy of annual legumes appeared to be risky, due to unfavorable production conditions during the summer period. However, annual green manure crops could have a higher potential than reported here if management techniques are improved.

If green manure crops are not applied directly as fresh matter, ensiling or drying is needed for preservation, which reduces the economic competitiveness. Therefore, the timing between cutting and application of green manure crops to sales crops is the key, as shown by a recent study in which clover was produced in the alley between rows of blackcurrants (Hefner et al., 2021).

5 | CONCLUSIONS

This study demonstrated that the N production of green manure increased with increasing number of cuts, and that the perennial green manure crops alfalfa, red clover, and white clover could produce 300–640 kg N ha⁻¹ per year. Furthermore, a low C:N ratio could be obtained, which facilitates the fast net mineralization of nitrogen from plant-based fertilizers. The highest N productions were obtained by perennial alfalfa, grass clover, and red clover cut at early and intermediate developmental stages. Re-cut was not possible with the annual green manure crops broad bean, lupine, and pea that produced 110–330 kg N ha⁻¹.

The biomass, N offtake, and N recovery of white cabbage and leek fertilized with green manure crops were comparable to poultry manure, indicating that green manure crops had positive effects on crop growth beyond that of N nutrition. In general, alfalfa and white clover showed a higher fertilizer value compared to red clover. The fertilizer value was enhanced by soil incorporation compared to surface application and showed higher soil N_{min} levels, indicating better synchrony between N supply from the plant-based fertilizer and N demand by crops.

Our study confirms perennial green manure crops are efficient fertilizers for N demanding organic vegetable crops, representing a strong alternative to animal-based ones. In conclusion, our study provides a basis on which to further restrict the use of animal-based fertilizers from conventional farming in organic vegetable production.

ACKNOWLEDGMENTS

This study was funded by the *Danish Ministry of Environment and Food* with contributions from the *GUDP/RDD5 project #34009-19-1563 ComCrop* under the *International Centre for Research in Organic Food Systems*.

DATA AVAILABILITY STATEMENT

The datasets analyzed in the current study are stored on the Organic eprints database #43242 and will be available upon reasonable request to the authors.

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How to cite this article: Lyng, M., Kristensen, H. L., Grevsen, K., & Sorensen, J. N. (2023). Strategies for high nitrogen production and fertilizer value of plant-based fertilizers. *Journal of Plant Nutrition and Soil Science*, 186, 105–115. <https://doi.org/10.1002/jpln.202200031>