Effects of C : N ratio in cut-and-carry green manure and nitrogen application rate in organic potato production

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Preface

This study was conducted at the Farming System Ecology group as part of my MSc thesis, which is fundamental part of my master in “Plant Sciences” at Wageningen University.

The purpose of the study was to investigate the effects of different C : N ratios in cut-and-carry green manures on an organic potato crop in The Netherlands. In order to achieve this, a field experiment was carried out and the influence of four green manure C : N ratios and three fertilizer rates on tuber yield, tuber quality, nitrogen, phosphorus and potassium dynamics were analysed.

The completion of this research was possible just with the contribution of some people. First of all, I thank my supervisor Egbert Lantinga who guided me through the whole study and Johannes Scholberg who helped with his precious advises. Likewise, I am thankful to my colleagues (Pauline Martel and Jean Claude Majuga), Andries Siepel, John van der Lippe, Wim van der Slikke and the rest of the staff from Unifarm and Hennie Halm from FSE for the laboratory analysis. Moreover, last but not least, I thank my family and friends in Italy for moral help and support.

Abstract

Potato is a worldwide important staple crop and due to its high value, it is one of the main crops grown in Dutch organic agriculture. However, organic agriculture in The Netherlands is still developing slowly compared to other European countries. Dutch organic farmers are facing a dependency on organic animal manure and often, transport costs make it economically infeasible. Cut-and-carry fertilizer practice, by means of mobile green manure application, reduces the dependency of farmers to external inputs. However, green manure nutrient composition is influenced by many characteristics like fertilizer regime, botanical composition and mowing time. In this study, we focus on C : N ratio of grass-clover silage and its affects yield and quality of potato tubers. In order to achieve this, a field experiment was carried out applying a mixture of grass-clover green manure to an organic potato crop. The two factors tested were four C : N ratios (24, 22, 17, 16) and three fertilization rates (57, 113, 170 Kg N ha^{-1}). We tested the effect on tuber yield, quality and tuber size, nitrogen, phosphorus and potassium content in soil, aboveground material and tubers. Simultaneously, a second experiment was carried out by burying 1 mm mesh bags filled with the four C : N ratio treatments. The purpose of this second experiment was to investigate N, P and K release over time from the four silages. The highest fresh tuber yield (i.e. 50.1 ton) was performed by the combination of lowest C : N ratio (i.e. 16) and the highest fertilization rate (i.e. 170 kg N ha^{-1}). At 10 weeks after planting, N accumulation was negatively affected by C : N ratio higher than 22. Crop performance indicators like ANR and PFP were not influenced by the C : N ratio of silage. Likewise, C : N ratio of the silage did not affect tubers quality. The silage bags experiment showed a faster initial N, P and K release by the lowest C : N ratio (i.e. 16). However, all four treatments released approximately 80% of the N after 14 weeks. This study confirms the effectiveness of grass-clover application for agricultural purpose in the Netherlands. Furthermore, it gives scientific evidence about the positive effect of low C : N ratio in green manure on fresh tuber yield production.

Keywords: *Solanum tuberosum*, organic, C : N ratio, green manure, grass-clover, tuber yield, tuber quality, nitrogen release, fertilization rate

Abbreviations

ANR: Apparent nitrogen recovery

C: Total carbon

DM: Dry matter

K: Potassium

N: Nitrogen

N_{min}: Available mineral nitrogen

N_{tot}: total nitrogen

P: Phosphorus

PFP: Partial Factor Productivity

SOM: Soil organic matter

TSG: Tuber Specific gravity

WAB: Weeks after burying

WAP: Weeks after planting

1. Introduction

1.1 Fertilizer, nitrogen and environment

The human population is growing steeply and agriculture is challenged to fulfil the food demand minimizing its impact on the environment. To overcome this problem, animal manure is widely used to improve soil properties and enhance crop performance. According to the World Resources Institute (2005), the production and use of animal manure causes one-third of total methane (CH_4) emissions. Despite methane forms only 15% of the total greenhouse gasses emissions, the global warming potential of CH_4 is 25 times higher than carbon dioxide (CO_2) (World resource institute, 2005). Moreover, ammonia (NH_3) volatilization is another main pollutant produced by the intensive use of animal manure. Bouwman *et al.*(2002) estimated a worldwide loss of 6.9 – 8.6 tons of N per year through NH_3 volatilization. Furthermore, nitrogen (N) losses may occur by leaching. Although the percentage of N leakage is generally rather low (10-13% circa of N volatilized) the effect of nitrates in the ground is relevant in terms of water pollution (Dewes, 1995). For this purpose, the *EU nitrates directive* set strict limitations on N fertilizer application and this policy is still valid for both conventional and organic agriculture (Anon., 1991).

1.2 Organic potato production in the Netherlands

Potato (*Solanum tuberosum* L.) is the fourth staple crop produced in the world with production of 368 million of tonnes on an area of 19.4 million of hectares (FAOSTAT, 2013). In The Netherlands, potato is the first crop in terms of production (i.e. 6.8 million of tonnes) with an approximately twofold yield per hectare compared to the average European production (i.e. 43 ton ha^{-1} and 19 ton ha^{-1}) (FAOSTAT, 2013). Important reasons for such a high production are the easy accessibility of water and fertilizer and a constant improvement of the cultivars by the breeding companies (Harris, 2012). However, organic agriculture is practised on a relatively small area (2.6% of total Dutch agricultural soil), which is less than a half compared to the European mean (i.e. 6.9%) (Eurostat, 2012). This scarcity is due to several bottlenecks, which make organic agriculture unprofitable for the farmer. Firstly, the Netherlands are known for a surplus in conventional animal manure but a lack in organic animal manure. Furthermore, livestock farms are mostly located on peaty soils not suitable for crop production (Sukkel & Hommes, 2009). This makes transportation of organic animal manure another obstacle for farmers. In facts, studies demonstrated that transportation increases manure price significantly. For instance, a model developed in Louisiana (United States) shows an optimal cut-off distance of 30 km for animal manure (Paudel *et al.*, 2009). Although this model was not made for the European market, a Dutch farmer has to produce his own fertilizer or to buy it nearby in order to achieve a reliable profit. Furthermore, Loncarevic *et al.* (2005) concerns about contamination in organic products by pathogens like *Escherichia coli* and *Listeria monocytogenes* after animal manure application. In addition, according to de Ponti *et al.* (2012) further bottlenecks are the limited demand of organic products, a higher unpredictability of the yield and the challenges associated with the conversion of a conventional to an organic farm.

1.3 Cut-and-carry fertilizer

In the last 10 years, many studies were carried out to find an alternative to the common fertilizers. Nowadays, the “cut-and-carry” practice with green manure is a valid substitute to the animal manure. Recent studies on potato (Litsos, 2015; Drakopoulos, 2014), spinach (Van der Burgt, 2011) and cauliflower (Aloysius, 2013) have shown higher or similar effect on crop yield by applying green manure, compared to animal manure (poultry and solid cattle manure). However, Litsos (2015) recommended to apply silage with a C : N ratio lower than 20 to avoid N immobilization. In order to have a low C : N ratio, cut-and-carry green manure should include leguminous species with high leaf : stem ratio or be mowed during the early stages of the crop (Sorensen & Thorup-Kristensen, 2011). Thorup-Kristensen (2006) showed that yield and N uptake of carrot, cabbage and lettuce was positively affected by leguminous species in green manure application. In the Netherlands, a mixture of Italian ryegrass (*Lolium multiflorum*) and white clover (*Trifolium repens*) is a common winter cover crop. When these two species are grown together they combine their positive benefits enhancing soil structure and stimulating soil biota community (van Eekeren, 2009).

The effect of different C : N ratio in green manure fertilizer on a full potato crop cycle is still poorly studied and its influence on the yield is unknown. In fact, due to the many factors involved in N immobilization (e.g. recalcitrant compounds in the litter, microorganism population etc.) (Fog, 1988), the effect of high C : N ratio amendments is still unpredictable. In order to fulfil this knowledge gap, a field experiment was carried out growing a potato crop applying grass-clover cut-and-carry fertilizer at low and high C : N ratios.

1.4 N, P, K release in plant based material

Nutrients released from animal manure have been amply studied under different climate conditions and, therefore, the N, P, K dynamics are mostly well known (Lehmann, 2003; Esse, 2001.). The purpose of these studies was to improve the efficiency of input applications reducing losses and enhancing the outputs. On the contrary, due to a scarce utilization, plant based fertilizers are still poorly studied in Europe and other temperate climates locations. However, studies on tropical legumes species (Cobo, 2002; Palm & Sanchez, 1991) and straw in China (Zhuang, 2001) have shown the influence of the chemical composition of plants material on nutrients release. In these studies N and P release was influenced negatively by high C : N, lignin : N and polyphenols : N ratios.

In a potato crop, the 3 main macronutrients are known to be nitrogen (N), phosphorus (P) and potassium (K) and their effect on yield and quality of the tubers is well studied. N deficiency can limit yield, whereas excessive N can leach to groundwater and cause environmental issues(Errebhi, 1998). Regarding P, an P fertilizer application enhances tuber biomass (Rosen, 2008). However, P influence on tuber quality as specific gravity and tuber dry weight is still controversial (Zelalem, 2009). Similar considerations are stated in previous studies concerning K effect in a potato crop. K application rate between 150 – 210 kgK ha^{-1} led to the best tuber yield (Allison, 2001). However, different experiments about the effect of K on tuber quality (i.e. starch content and tuber specific gravity) gave contrasting results (Khan, 2012; Westermann, 1994).

As mentioned above, the release of these key nutrients in temperate climate by plant residues is still poorly studied. Therefore, another experiment was carried out, burying grass-clover green manure in litterbags into the potato ridges to assess N, P, K release.

2. Purpose of the study

2.1 Research aim

The aim of this study is to investigate the effect of different C : N ratios in green manure amendments on tuber yield and tuber quality on organic potato production. Furthermore, N, P, K released the silage were studied concurrently through a litter bag experiment.

2.2 Research questions

i) Do high C : N ratios (i.e. 22, 24) lead to N immobilization and a reduction in the yield?
ii) Which C : N ratio will perform better in terms of final tuber yield?
iii) Do high C : N ratios increase tuber quality in terms of starch content and specific gravity?
iv) Will C : N ratio affect N accumulation during the late stages of the crop?

2.3 Hypotheses

According to Joern *et al.* (1995), N application rates that are higher than 112 kgN ha^{-1} enhance tuber yield. However, many studies on potato (Westermann, 1994; Drakopoulos, 2014) show a negative influence of high N applications on tuber quality (lower specific gravity and starch content). Therefore, our hypotheses are:

I. Due to the high responsiveness of potato to N, higher application rates will lead to an increasing tuber yield.
II. Higher N application will reduce the starch content in the tubers and, therefore, will cause a lower specific gravity and starch content.
III. High C : N ratios (i.e. 24 and 22) in fertilizer amendments will lead to reducing N uptake by the crop.
IV. A faster N release from the fertilizer is expected at low C : N ratios.

3. Materials and methods

3.1 Experimental location, field history and climatic conditions

A field experiment was carried out between April and September 2015 on the organic experimental farm of Droevendaal (51°59'33.68"N, 5°39'34.59"E), the organic certified research facility of Wageningen University and Research in Wageningen in the Netherlands. The soil texture of the experimental site is classified as sandy. In particular, the last soil texture analyses (2013) show a clay, silt and sand content of, respectively, 2%, 10% and 85%. Soil initial state (Table 3) was analysed before the experiment and it showed an initial SOM content of 3.6%. Moreover, the soil had an average pH-H_2O value of 6.2 and a pH-KCL of 5.2.

Before this experiment spring barley (*Hordum vulgare* L.) was grown and during the winter a mixture of mustard and radish was grown as cover crop.

The mean yearly temperature of the last 10 years was 10.4°C and a yearly cumulative rainfall of 655 mm. Rainfall (mm), weakly minimum and maximum temperature (°C) during the potato crop cycle were collected from the local weather station (Fig. 1). During the crop cycle the cumulative rainfall was 216.4 mm, the mean of maximum and minimum temperatures were 20.4°C and 10.9°C respectively.

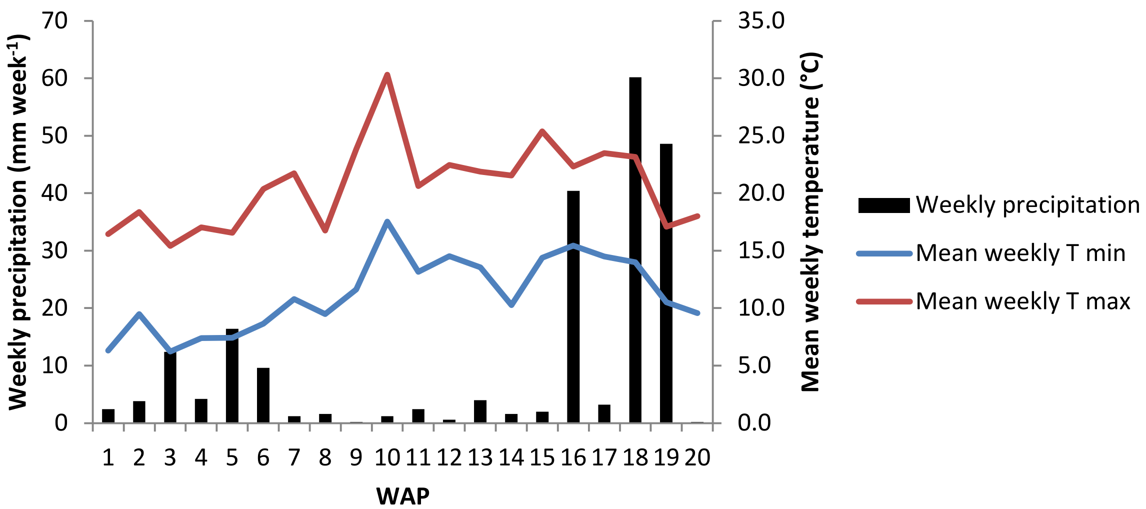

Figure 1. Cumulative weekly precipitation (mm week^{-1}), minimum (T min) and maximum (T max) temperature (°C) during potato crop cycle.

3.2 Experimental setup

The experimental design was a completely randomized block design, which it is shown in the appendix figure 10. Every block was divided in 13 plots and replicated 4 times. 12 treatments were determined by 2 factors: C : N ratio of the silage and fertilizer application rate. The first factor entailed 4 C : N ratio levels (24, 22, 17, 16), whereas the second factor consisted of 3 application rates (57, 113, 170 kgN ha^{-1}). In addition, a treatment without fertilizer application was included as a control. The higher application level (i.e. 170 kgN ha^{-1}) was considered in order to fit in the limitation on N usage in agriculture imposed by the *EU directive nitrates* (Anon., 1991) for animal manure and chemical fertilizer. However, a lack of regulation in this policy allows a fertilizer application of plant residues higher than 170 kgN ha^{-1}. The combination between the two experimental factors are listed in table 1. Every plot measured 15 m × 3 m = 45 m^2 and composed by 4 ridges. The total experimental surface was 2500 m^2 circa. Every plot was subdivided in 3 parts: an external part, an inner part for pre-harvest destructive measurements and a net plot designed for the final harvest (Appendix Fig. 11).

Table 1. List of 12 treatments + control

Treatment	C : N ratio	Application rate (kgN ha^{-1})
F1	24	57
F2	24	113
F3	24	170
F4	22	57
F5	22	113
F6	22	170
F7	17	57
F8	17	113
F9	17	170
F10	16	57
F11	16	113
F12	16	170
F13 (control)	-	-

3.3 Agronomical practices

In 2014, a mixture of Italian ryegrass (*Lolium multiflorum*) and white clover (*Trifolium repens*)was grown as a cover crop on a different area nearby the experimental field. This cover crop was cut four times between May and November. Plant material was dried on the field and then wrapped in bales with a plastic film in order to preserve it during the winter. In April 2015, DM (%), ash content (%), total C, N, P and K (%) were measured in the silage obtained from the four cuts. Using those values, the correct application rate and C : N ratio were determined. Total C content was determined using the Dumas Method with a CHN1110 Element Analyser (CE instruments, Milan, Italy). The results are shown in table 2. Further methodologies for these measurements are fully described in chapter 4.3.

On 13th of April, the winter catch-crop was mowed and incorporated into the soil. Three days later, silage amendments were chopped and applied manually to the field. The next day, silage was incorporated into the soil by means of a 15 cm deep ecoplough by a shallow mouldboard machine. On 22nd of April tuber seeds were sown by a potato planter in 4 rows per plot and the ridges were created. The distance between the potato rows was 75 cm and the distance between plants was 29 cm. The total plant density was 4.6 plants m^{-2}. The distance between the plants was suggested by the breeding company in an information leaflet (Agrico, 2015). During the crop cycle, every row was re-ridged at 7 weeks after planting (WAP). In the same week, weeds were removed mechanically thanks using an harrow machine. At 14 and 15 WAP, weeds were removed manually. At 18 WAP, the above ground material (leaves and stems) was burnt in order to facilitate the final harvest and sterilize the field from any pest infection or parasite infestation. At 10 WAP, Colorado potato beetle adults

(*Leptinotarsa decemlineata*) were observed on the experimental field From that date, their presence was monitored during the entire experiment. The beginning of the infestation was relatively late, and the harvest occurred before the hatching of the second generation.

On the 10th and 11th of September, the two inner rows of each plot (green inner area in fig. 11) were harvested manually and tubers were stored in labelled net bags in university facilities. The harvested tubers were employed for the final harvest measurements.

In 2014, on the same experimental site, an experiment on organic potatoes was spoiled by a *Phytophtora infestans* infestation and stopped at 11 WAP (Litsos, 2015). In order to reduce the chances of a blight infestation, an extremely *Phytophtora* resistant variety was needed. Therefore, the cultivar used was "Carolus" new cultivar bred by the company Agrico in the Netherland. This variety is assumed to be immune to both tuber and foliage late blight (Agrico, 2015).

Table 2. Harvest time, Dry matter (DM), ash content, total Carbon (C), total nitrogen (N_{tot})(%) and C : N ratio (-) of the four cuts.

	DM (%)	Ash (%)	C (%)	N_{tot} (%)	C : N (-)
Harvest time					
May	73	9	43.6	1.8	24
Jun-Jul	64	9	44.2	2.0	22
Aug-Sep	47	11	46.0	2.7	17
Oct-Nov	19	22	41.2	2.6	16

3.4 Silage nutrients release experiment

Simultaneously with the main field experiment a second experimentation was carried out in order to investigate N, P and K release from grass-clover plant residues. As shown in the experimental design (Appendix, Fig. 10), one plot per block was left without any treatment. Six litter bags containing silage from one of the different C : N ratios were buried 30 cm deep into the ridges. The total amount of litter bags buried into the soil was: 6 × 4 treatments × 4 blocks = 96 litter bags. Every bag measured 30 cm × 20 cm. Each of them was filled with 30 grams circa of fresh silage and sealed up by a plastic stopper. The matrix of the net used as material to make the bags was 1 mm. At 3 WAP, 24 litter bags per block were buried among potato plants at a distance of 2.5 m. At 1, 2, 4, 8, 12 and 14 weeks after burying (WAB), one sample of each silage type was taken from every plot.

4. Measurements and analysis

4.1 Pre-experimental analysis

Before the fertilizer was applied, the initial status of the 0 - 30cm soil layer was determined through the following procedure. Ten samples per block were collected following a zig-zag pattern by means of a soil gouge. These samples were then mixed in order to form a single sample. Hence, from every sample a representative sub-sample was analysed to determine ash content (%) dry matter (% DM), soil organic matter (%), pH, available mineral nitrogen (N_{min}), P-PO_4 and K. Ash content and organic matter were measured using the loss-on-ignition (LOI) methodology through combustion of the samples in a furnace at 500-550 °C for 3 hours (Konare *et al.* 2010). The loss in weight is the organic matter whereas the residues left from the sample are the ashes. NO_3^- and NH_4^+ and P-PO4 were determined following the methods described in Houba *et al.* (2000). Samples were dried at 40 ^{o} C, extracted in 0.01M calcium chloride and analysed using a segmented-flow system (Auto-analyzer II, Technicon). From the same sample, K was measured with a Varian AA240FS fast sequential atomic absorption spectrometer (Terneuzen, the Netherlands). After 0.01M calcium chloride was added to the soil samples pH was measured using a pH/mV meter (Inolab pH/Cond Level 1, WTW, Weilheim, Germany). The results (Table 3) show an adequate starting N, P and K content.

Table 3. Soil organic matter (SOM), Available mineral nitrogen (N_{min}), phosphorus (P-PO_4) potassium (K) and pH-H_2O of 0-30cm soil layer before planting.

	SOM (%)	N_{min} (kgN ha^{-1})	P-PO_4 (kgP ha^{-1})	K (kgK ha^{-1})	pH-H_2O (-)
block					
1	2.9	3.2	4.0	184	6.3
2	3.8	3.0	1.5	187	6.1
3	3.8	3.3	1.6	168	6.1
4	3.8	3.2	1.2	166	6.1
average	3.6	3.2	2.1	176	6.2

4.2 Soil N, P, K

At 6, 12 and 20 WAP, 20 soil samples per plot from the 0-30 cm soil layer were collected with a soil gouge following a zig-zag pattern. Samples from each plot were mixed and a 100 g and a 30 g sub-samples were taken. The first was dried in an oven at 105^o C for 24 hours in order to measure soil DW (%). The latter was analysed fresh in order to measure available N-NO_3^-, N-NH_4^+, P_2O_5 and K. The methodology used to extract the nutrients is the same as mentioned in 4.1. N-NO_3^- and N-NH_4^+ were summed in order to obtain available mineral nitrogen content (N_{min}).

4.3 Tuber yield, quality, size and tuber and plant N, P and K content

At 10 and 14 WAP two plants per plot were harvested from the part of the plot used for destructive measurements (red area in fig. 11). Shoot and tuber dry weight (%) were determined drying samples in an oven at 105^o C for 48 hours. Tuber and shoot samples were chopped, dried at 70^o C for 72 hours and grinded in 1 mm long pieces in order to determine total N, P and K content. Samples were digested adding a mixture of H_2SO_4–Se and salicylic acid (Novozamski *et al.*, 1983). In these digests total N and P were measured spectrophotometrically with a segmented-flow system (Auto-analyzer

II, Technicon). In the same samples K was measured with aVarian AA240FS fast sequential atomic absorption spectrometer (Terneuzen, the Netherlands).. At 20 WAP, the manual harvest was carried out collecting tubers from the green area shown in figure 11. Tuber fresh weight of each plot was measured in order to determine the total tuber fresh weight (ton ha^{-1}). Furthermore, from a 15 kg subsample tubers size was classified in three categories: small (15-40 mm diameter), large (>40 mm diameter) and cull (tubers damaged, smaller, infested or green) through a 0 – 15 cm and a 0 – 40 cm sieves. This potato variety is known to be rather susceptible to common scab in light soils (Agrico, 2015) therefore, a substantial amount of cull potatoes was expected. Marketable yield (ton ha^{-1}) was calculated as:

Marketable yield = Total tuber fresh yield – Cull tuber yield.

Afterwards, a 5 kg sample from the marketable yield of every plot was collected to measure the tuber specific gravity (TSG) applying the following formula:

$TSG = W_{air} / (W_{air} - W_{water})$.

Whereas W_{air} is the tuber weight in air and W_{water} is the tuber weight in water.

TSG was used to calculate starch content in tubers thanks to the formula given by Simmonds (1977):

Starch content = -1.39 + 0.196 [1000 × (TSG - 1)].

Two further tuber subsamples of 0.5 kg and 0.1 kg were collected in order to determine respectively tuber DM (%) and total N, P and K content. The methodology to measure DM and total N, P, K is the same as mentioned before in this section.

4.4 N accumulation, Apparent N Recovery (ANR) and Partial Factor Productivity (PFP)

N accumulation in plants will be calculated using the formula:

$(DM_{shoot} \times N\ content_{shoot}) + (DM_{tuber} \times N\ content_{tuber})$.

Apparent N recovery was calculated from N accumulation as:

$ANR\ \% = 100 \times (N\ accumulation_{treatment} - N\ accumulation_{control}) / N_{application}$.

Partial Factor Productivity (PFP) was determined:

$PFP = (Yield_{treatment} - Yield_{control}) / N_{applied}$.

4.5 Silage nutrients release

Every sample was dried at 70 ^{o} and grinded in 1 mm long pieces. Total N, P and K were extracted from the grinded material as described in 4.3.

After the samples collection a contamination of soil particle and roots was noticed. Whereas the roots were removed manually, the soil contamination was corrected using the formula given by to Potthoff and Lofttfield (1998):

$SC = (AC_{AR} – AC_{BP}) / AC_{S}$.

Where SC is the dry weight of soil contamination (g), AC_{AR} is the ash content of plant material in litterbag (mg) after removal, AC_{BP} is the ash content of plant material in litterbag (mg) before its placement, and AC_S is the ash content of the soil (mg g^{-1}).

After the contamination by the soil was removed, nutrient released was determined through the following formulas:

$N_{released} = 100 - ((N_{treatment} \times 100) / N_0)$

Whereas, $N_{released}$ is the N released from the sample (%), $N_{treatment}$ is the N content of the sample collected (g), and N_0 is the initial N content of the sample (g).

$P_{released} = 100 - ((P_{treatment} \times 100) / P_0)$

Whereas, $P_{released}$ is the P released from the sample (%), $P_{treatment}$ is the P content of the sample collected (g), and P_0 is the initial P content of the sample (g).

$K_{released} = 100 - ((K_{treatment} \times 100) / K_0)$

Whereas, $K_{released}$ is the K released from the sample (%), $K_{treatment}$ is the K content of the sample collected (g), and K_0 is the initial K content of the sample (g).

5. Statistical analysis

Data were statistical analysed using GenStat (VSN International Ltd., Hemel Hempstead, UK) 17^{th} edition version 17.1.0.14713 (64-bit) through an analysis of variances (ANOVA). Initially, a Shapiro-Wilk test was used to determine the normal distribution and homogeneity. Natural logarithm was calculated for non-homogeneous or non-normal distributed values. Difference between the treatments was assessed and separated by Fisher's protected LSD-test. Analysis of variances of C : N ratio factor and interaction C : N ratio (F) × Fertilization rate (R) was conducted excluding control treatments. Control was included in the ANOVA for Fertilization rate factor. Both Shapiro-Wilk and LSD tests were validated by $P<0.05$.

6. Results and discussion

6.1 Tuber yield – Fresh, Dry weight and tuber DM

At 10 WAP, no significant difference between the treatments was found in fresh total yield under any factor due to a quite high variation in the results (Table 4). A relevant result is that the highest C : N ratio performed similarly to the control (4 and 4.1 ton ha^{-1}, respectively). We observed a large variation in plant emergence and size between plants in the same plot. Moreover, the number of plants harvested was rather low (i.e. two plants per plot). Therefore, it is likely to state that the these two causes are the main reason for such variation in the data. At 14 WAP, the difference between the C : N ratios was not significant (Table 4). On the other hand, the fertilization rate factor showed a considerable variance in fresh tuber yield (Table 4). Under the highest fertilization rate treatment (170 kgN ha^{-1}) potato plants had twofold tuber yield production compared to the control (54 and 27 ton ha^{-1} respectively) (Table 4). Many treatments show a higher yield at 14 WAP than the final harvest (20 WAP) (Table 4). In this case also, we collected two plants per plot and this low pool of data might have led to an overestimation of the yield. However, it is relevant to remark an averagely six fold increase in tuber biomass in solely four weeks (Table 4). Those weeks were characterized by higher temperatures (i.e. 19.3°C) (Fig. 1) compared to the Dutch historical average (i.e. 16.7°C) (AmbiWeb GmbH, n.d.). Although potato is a C3 crop, its life cycle and yield are responsive to high temperatures under optimal conditions (Pereira, 2008). Hence, these high temperatures might have improved N uptake, enhancing tuber biomass and N accumulation (Tables 4 and 9). In addition, based on field observations, the crop has not shown any visible water deficiency during the whole cycle. Thus, it is likely that the crop grew under close to optimum conditions. The final harvest showed a little difference in tuber yield between the lowest C : N ratio (i.e. 16) (43.6 ton ha^{-1}) and the two highest (i.e. 24, 22) (39.9 and 39.3 ton ha^{-1} respectively) (Table 4). To answer our second research questions (ii), C : N ratio 16 performed the best in terms of final tuber yield. Therefore, C : N ratio in grass-clover silage have a On the contrary, the effect of N application on yield was evident. Table 4 shows an increase of 5-7 ton ha^{-1} in tuber yield by applying additional 57 kgN ha^{-1}. Therefore, the highest fertilization rate had the best performance (47.5 ton ha^{-1}) and the increase in yield was higher than 60% compared to the control (30.5 ton ha^{-1}). These findings confirms our hypothesis (I) where N fertilization rate and tuber yield are directly related. These findings disagree with Litsos (2015) and Drakopoulos (2014) where N applications higher than 113 kgN ha^{-1} did not increase the final tuber yield. However, in these two studies late blight infection was reported and it might have limited the final tuber yield.

In terms of tuber dry matter content (DM) (%), no significant difference between C : N ratio treatments was found in all the harvests (Table 5). On the other hand, tuber DM was influenced by th fertilization rate factor. At 14 WAP the highest fertilization rate (i.e. 170 kgN ha^{-1}) led to a lower tuber DM compared to the other rate treatments (Table 5). At the final harvest, the highest fertilization rate shows DW content of 19 % (Table 5). This value fits in the interval (i.e. 18-20 %) given by Gravoueille (1997) for marketable tubers employed in fresh consume. DM Yield was not influenced by the C : N ratio of the silage applied (Table 5). At 14 WAP and at the final harvest it was highly affected by the fertilization rate (Table 5). The highest rate performed the best (9 ton ha^{-1}) with a yield almost 50% times higher than the control (6.2 ton ha^{-1}) whereas, rates in between gave intermediate values.

Table 4. C : N ratio (24, 22, 17, 16) and fertilization rate (0 (Control), 57, 113, 170 kgN ha^{-1}) effect on fresh total yield at 10, 14 and 20 WAP (Final harvest) and Marketable yield (ton ha^{-1}). Different letters refer to significant differences according to Fisher's protected LSD-test (P<0.05).

	Fresh total yield (ton ha^{-1})			Marketable yield (ton ha^{-1})
	10 WAP[1]	14 WAP	Final harvest	Final harvest
C : N Ratio (F)				
24	4.0	47.4	39.9 b	37.7 bc
22	7.9	42.8	39.3 b	37.3 c
17	9.3	46.1	42.4 ab	40.7 ab
16	8.7	44.4	43.6 a	41.3 a
Significance[2]	ns	ns	*	*
Fertilization rate (R)				
0 (Control)	4.1	27 c	30.5 d	29.1 d
57	5.6	37 bc	35.8 c	33.9 c
113	9.0	45 ab	40.5 b	38.5 b
170	7.7	54 a	47.5 a	45.4 a
Significance	ns	***	***	***
F x R	ns	ns	ns	ns

[1] WAP= Weeks after planting

[2] *, ** and *** refer to *P* values < 0.05, < 0.01 and < 0.001, respectively; ns = not significant.

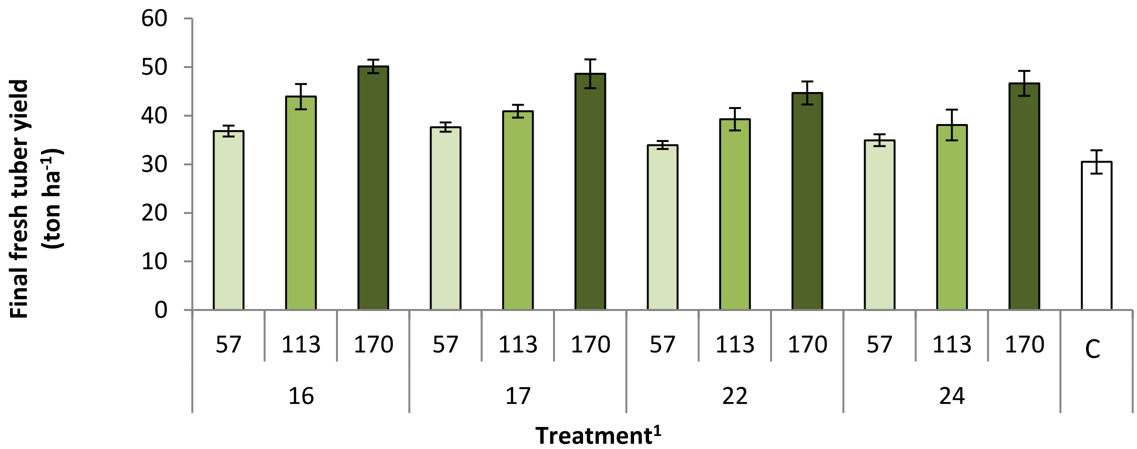

Figure 2. Final fresh tuber yield (ton ha^{-1}) influenced by Fertilization rate (0 Control (C), 57, 113, 170 kgN ha^{-1}) and C : N ratio (16, 17, 22, 24) at 20 WAP (Final harvest).. Error bars represent standard error.

[1]Top line of treatment refers to fertilization rate, bottom line refers to C : N ratio

Table 5. C : N ratio (24, 22, 17, 16) and fertilization rate (0 (Control), 57, 113, 170 kgN ha^{-1}) effect on tuber dry matter (%) and tuber dry matter yield (ton ha^{-1}) at 10, 14 and 20 WAP (Final harvest). Different letters refer to significant differences according to Fisher's protected LSD-test (P<0.05).

	Tuber DM (%)			**Tuber DM Yield (ton ha^{-1})**		
	10 WAP[1]	14WAP	Final harvest	10 WAP	14 WAP	Final harvest
C : N Ratio (F)						
24	8.8	18.1	19.8	0.4	8.4	7.9
22	8.5	18.5	19.4	0.7	8	7.6
17	8.2	19.2	19.6	0.8	8.8	8.3
16	6.8	18.9	19.5	0.6	8.4	8.5
Significance[2]	ns	ns	ns	ns	ns	ns
Fertilization rate (R)						
0 (Control)	10.7	20.6 a	20.4 a	0.4	5.5 c	6.2 d
57	8.3	19.4 a	20.2 ab	0.5	7.2 bc	7.2 c
113	7.7	18.3 b	19.6 a	0.7	8.3 ab	7.9 b
170	8.2	18.3 b	19.0 c	0.6	9.7 a	9.0 a
Significance	ns	**	***	ns	**	*
F x R	ns	ns	ns	ns	ns	ns

[1] WAP= Weeks after planting

[2] *, ** and *** refer to *P* values < 0.05, < 0.01 and < 0.001, respectively; ns = not significant.

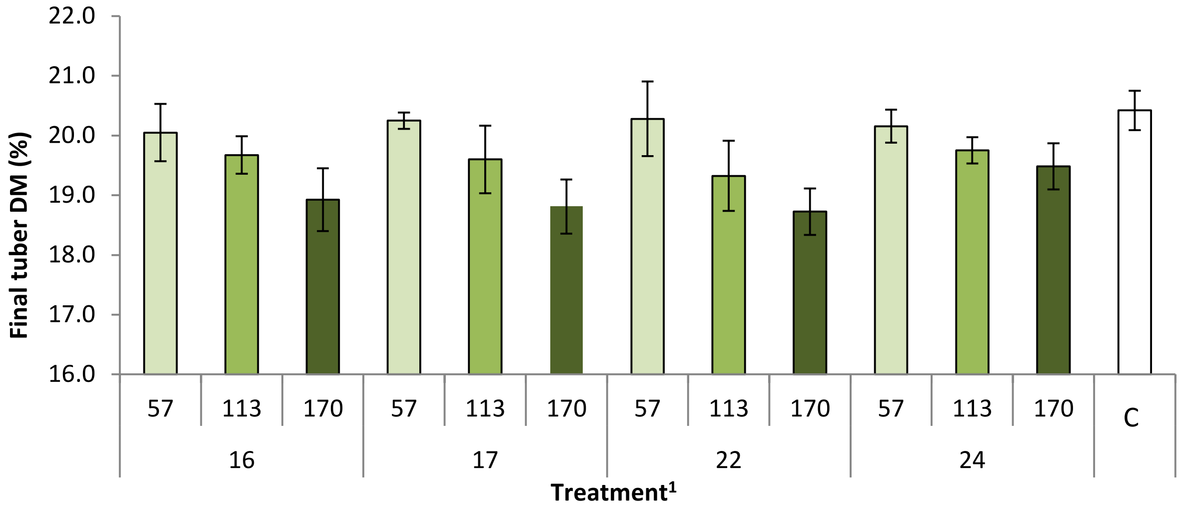

Figure 3. Final DM content in potato tubers (%) influenced by Fertilization rate (0 Control (C), 57, 113, 170 kgN ha^{-1}) and C : N ratio (16, 17, 22, 24) at 20 WAP (Final harvest). Error bars represent standard error.

[1]Top line of treatment refers to fertilization rate, bottom line refers to C : N ratio

6.2 Tuber quality – Starch, specific gravity and tuber category

As mentioned in chapter 4.3, tuber specific gravity was considered to affect directly the starch content of the tubers according to the formula given by Simmonds (1977). Therefore, the next statements concerning starch can be considered valid also for the tuber specific gravity.

Previous studies regarding the effect of N on potatoes showed a negative effect of high application rates on tuber starch content due to an increase of the water content in the tissues (Westermann, 1994). In fact, the plants uptakes more water in order to maintain the cell turgor decreased by the higher solution potential (Westermann, 1994). Table 6 shows a similar tendency, since the starch content (%) of the highest fertilization rate was lower than the other treatments. Hence, starch content in the tubers was reduced by high fertilization rates, confirming our second hypothesis (II). Table 5 and 6 show that starch content and tuber DM have similar trend. Studies carried out in the past (Kadam, 1991; Alva, 2007) have shown that starch forms the majority of the tuber DW and there is a negative correlation between N application, starch content and DM %. On the contrary, C : N ratio of the silage did not influence tuber specific gravity and starch content (Table 6). Hence, this answer our fourth research question (iv) since quality of the tubers was not affected by the fertilizer C : N ratio. This result agrees with Drakopoulos (2014), where the effect on starch content by different C : N ratios (i.e. solid cattle manure, C : N ratio 12 and grass-clover silage, C : N ratio 22)was not significantly different. As mentioned before, N is strictly correlated to starch and at the final harvest most of the N contained in the silage residues was already released (Fig. 7). Basis for this statement can be found in the final total N accumulation (Table 9) and N released from silage residues over time (Fig. 8). At the final harvest, no significant difference in N accumulation between different C : N ratio treatments was found (Table 8) and approximately 80% of the N stored in all the different residues was already released (Fig. 8).

As shown in table 6, the difference in cull tuber weight was not significant under any factor. However, the fraction of cull potato was considerable high in every treatment (mean of 1.9 ton ha^{-1} cull tubers) (Table 7). Based on our observations, most of the cull yield was composed by green tubers. This might be due the superficial tuberization typical for the “Carolus” cultivar (Agrico, 2015). In addition, the height of the ridges was reduced by the abundant late-summer rainfall (Fig. 1), exposing several tubers to the light. On the contrary, tuber size was greatly affected by the N application rate (Table 7). Figure 5 shows an increase of 12-17% circa in large tuber fraction between the control and the highest N application rate. A similar trend is shown by Terra (2014) and Litsos (2015). Studies carried out in the past (Dahlenburg, 1990; Krentos, 1979) stated that tuber size is also influenced by environmental conditions, agronomical practices (e.g. seeding density) and intrinsic traits of the cultivar. This findings are useful for some specific potato production systems, where the tubers must fall in a certain length range in order to be considered marketable (e.g. tubers for processed products). Table 7 shows a slightly higher large tuber yield under the two lowest C : N ratios. According to Westermann (1985), N availability during both vegetative and tuberization phases enhances tuber bulking. N accumulation in table 9 shows that during the tuber initiation (10 WAP)more N was available under low C : N ratio.

Table 6. C : N ratio (24, 22, 17, 16) and fertilization rate (0 (control), 57, 113, 170 kgN ha^{-1}) effect on tuber specific gravity and tuber starch content (%) at 20 WAP (Final harvest). Different letters refer to significant differences according to Fisher's protected LSD-test (P<0.05).

	Tuber Specific Gravity (-)	**Starch Content (%)**
C : N Ratio (F)		
24	1.074	13.1
22	1.074	13.2
17	1.075	13.3
16	1.074	13.2
Significance[1]	ns	ns
Fertilization rate (R)		
0 (Control)	1.077 a	13.6 a
57	1.077 ab	13.6 ab
113	1.074 ac	13.2 ac
170	1.073 c	12.9 c
Significance	**	**
F x R	ns	ns

[1] *, ** and *** refer to *P* values < 0.05, < 0.01 and < 0.001, respectively; ns = not significant.

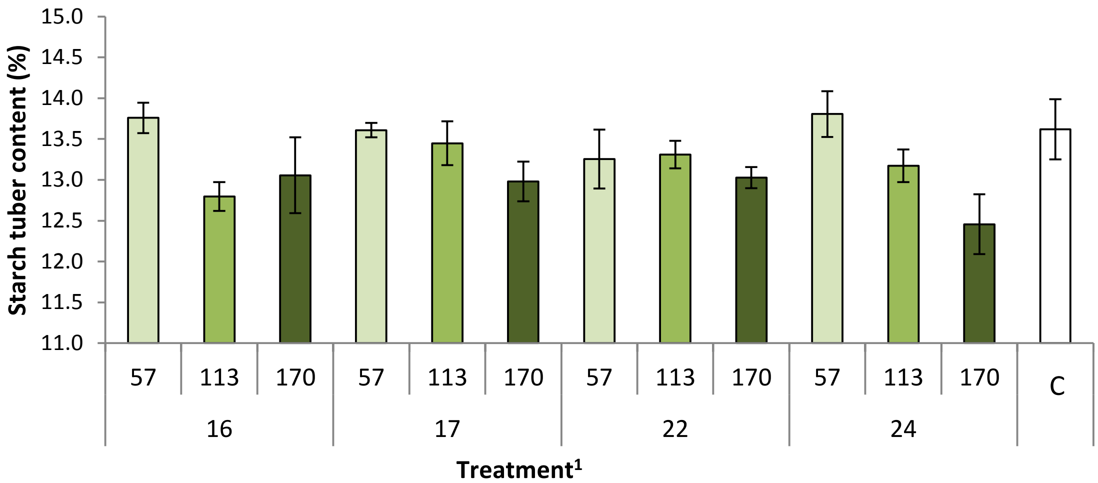

Figure 4. Starch content in potato tubers (%) influenced by Fertilization rate (0 Control (C), 57, 113, 170 kgN ha^{-1}) and C : N ratio (16, 17, 22, 24) at 20 WAP (Final harvest). Error bars represent standard error.

[1]Top line of treatment refers to fertilization rate, bottom line refers to C : N ratio

Table 7. C : N ratio (24, 22, 17, 16) and fertilization rate (0 (control), 57, 113, 170 kgN ha^{-1}) effect on yield of the 3 categories (ton ha^{-1}) at 20 WAP (Final harvest). Different letters refer to significant differences according to Fisher's protected LSD-test (P<0.05).

	Tuber Category (ton ha^{-1})		
	Large (> 40mm)	Small (15 < 40mm)	Cull
C : N Ratio (F)			
24	30.6 ab	7.1	2.2
22	29.8 b	7.5	2.0
17	33.7 a	6.9	1.7
16	33.8 a	7.5	2.3
Significance[1]	**	ns	ns
Fertilization rate (R)			
0 (Control)	21 d	8.5 a	1.4
57	26 c	8.2 a	1.9
113	31 b	7.2 ab	2.1
170	39 a	6.4 b	2.1
Significance	***	*	ns
F x R	ns	ns	ns

[1] *, ** and *** refer to *P* values < 0.05, < 0.01 and < 0.001, respectively; ns = not significant.

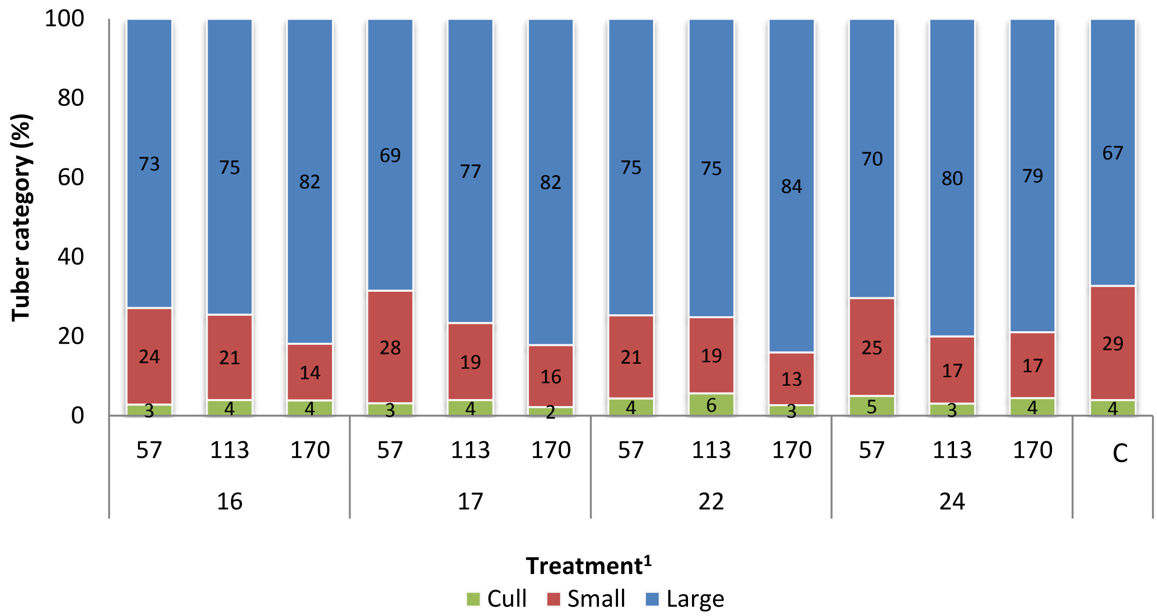

Figure 5. Small, Large and Cull tuber categories (%) influenced by Fertilization rate (0 Control (C), 57, 113, 170 kgN ha^{-1}) and C : N ratio (16, 17, 22, 24) at 20 WAP (Final harvest)..

[1]Top line of treatment refers to fertilization rate, bottom line refers to C : N ratio

6.3 Tubers and above ground N, P, K%

At 10 WAP, N content (%) in above ground material and tubers was not influenced significantly by both C : N ratio and fertilization rate factors (Table 8). These results agree with the visual observations taken during the crop cycle. No N deficiency was observed in the plants and a first yellowing of the leaves was noticed at 16 WAP. Despite the observations, a first general reduction in N content of the above ground material was measured 14 WAP (Table 8). This reduction is due to the significant increase in tuber weight between 10 and 14 WAP. Potato plants increase tuber weight translocating nutrients (e.g. N) . Although the homogeneity in the colour of the leaves, table 8 shows a slightly lower N content (%) of both shoots and tubers in the control and in the lowest application rate. At the final harvest, no significant difference in N content of the tubers was measured (Table 8).

At 10 WAP P content in leaves and tubers was not influenced by the two factors (Table 8). P content in grass and clover silage was relatively low and, due to the low P mobility, it needs a well-developed root system in order to be taken up by the crop. P is a key element during the early stages of the crop (Harris, 2012) and its availability is crucial. However, the values measured (0.4%) in the aboveground material are close to the optimal P content in the shoot (0.5%) given by MacKay *et al.* (1966). Therefore, it is likely that P was not a limiting growing factor in the crop.

Table 8 shows more than adequate content of K in aboveground material and tubers under every treatment. In fact, the optimum value in the shoot (i.e. 3.9 K%) estimated by MacKay (1966) was overcome by every factor including the control (i.e. 4.8%). Therefore, K also was not a limiting growing factor in the crop. This large quantity of K was caused by different factors. Firstly, we measured a relatively high K content in the soil (i.e. 176 kgK ha^{-1}) at the beginning of the experiment (Table 3). Secondly, temperatures and rainfall had a significant role in this high K availability. K is known to appear mostly in an unavailable crystalline form and it has to be converted by factors like soil moisture and temperature in order to become available. K needs water to move from the soil to the roots and it is taken up mostly by mass-flow and diffusion (Kuchenbuch, 1986). Lastly, figure 9 shows a relatively fast K release from the silage, where more than 90% of the total K was released after 14 weeks. K% in aboveground material and tubers was influenced by the fertilizer rate (Table 8). In the shoot, control has the lowest value and K content increases at higher fertilization rates. On the contrary, in the tubers just the control had a significantly lower K content compared to the other treatments.

Table 8. C : N ratio (24, 22, 17, 16) and fertilization rate (0 (control), 57, 113, 170 kgN ha^{-1}) effect on total Nitrogen, Phosphorus and Potassium content (%) in aboveground material and tubers at 10, 14 and 20 WAP (Final harvest). Different letters refer to significant differences according to Fisher's protected LSD-test (P<0.05).

	N aboveground (%)		N tuber (%)			P aboveground (%)	P tuber (%)	K aboveground (%)	K tuber (%)
	10 WAP[1]	14 WAP	10 WAP	14 WAP	Final Harvest	10 WAP	10 WAP	10 WAP	10 WAP
C : N Ratio (F)									
24	4.0	2.0	2.1	1.5	1.4	0.4	0.3	5.5	3.4
22	4.0	2.1	2.3	1.3	1.6	0.4	0.3	5.4	3.5
17	3.9	2.0	2.1	1.3	1.4	0.3	0.3	5.2	3.4
16	4.0	2.1	2.2	1.3	1.5	0.4	0.3	5.6	3.4
Significance[2]	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fertilization rate (R)									
0 (Control)	3.9	1.8 c	1.8	1.23 ab	1.4	0.4	0.3	4.8 c	2.8 b
57	3.9	2.0 b	2.1	1.20 b	1.4	0.4	0.3	5.1 bc	3.3 a
113	3.9	2.0 ab	2	1.40 a	1.4	0.4	0.3	5.4 b	3.4 a
170	4.2	2.1 a	2.3	1.44 a	1.6	0.4	0.3	5.8 a	3.5 a
Significance	ns	**	ns	*	ns	ns	ns	***	*
F x R	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1] WAP= Weeks after planting

[2] *, ** and *** refer to *P* values < 0.05, < 0.01 and < 0.001, respectively; ns = not significant.

6.4 N accumulation, N uptake, ANR and PFP

Table 9 shows a significant difference in N accumulation between C : N ratio treatments at 10 WAP. Higher C : N ratio led to a lower N accumulation in aboveground material and tubers. Total N accumulation by the highest (i.e. 24) C : N ratio (80 kgN ha^{-1}) was lower compared to the other treatments (105 ± 6 kgN ha^{-1}) (Table 9). This partially confirms our hypothesis (III) where high C : N ratio in amendments would lead to an immobilization of the N released from the silage. It is partially confirmed because N immobilization was expected also under 22 C : N ratio treatment. At 10 WAP, Apparent Nitrogen Recovery (ANR) by C : N ratio 24 was relatively low (16 kgN ha^{-1}) compared to the other treatments (47.5 ± 5.5 kgN ha^{-1}) (Table 10). However at 14 WAP and at the final harvest (20 WAP), no significant difference was found between C : N ratios in N accumulation and ANR (Table 9 and 10). The latter result disagrees with the findings of Sorensen Thorup-Kristensen (2011) where a higher C : N ratio in the silage had a negative effect on ANR. Between 10 and 14 WAP, the N uptake performed by C : N ratio 24 was relatively high (Table 10) reaching the N accumulation values performed by the other treatments (Figure 6). In a previous study (Litsos, 2015), C : N ratio higher than 20 in grass-clover silage led to N immobilization. On the contrary, our results suggest N immobilization driven by C : N ratio higher than 22. Before 14 WAP, N was released straight available by the components of the soil involved in N immobilization. Therefore, to answer our fourth research question (iv), N was released far before the end of the crop cycle leading to homogeneous values in the final N accumulation.

Table 9 shows a different trend by the fertilization rate factor on N accumulation. Initially, at 10 WAP, the 2 highest rates (i.e. 113 and 170 kgN ha^{-1}) performed better than the control. Between 10 to 14 WAP, the highest rates had a higher N uptake compared to the other treatments (Table 10). In terms of total N accumulation, the difference between control and the lowest rate was not significant. At the final harvest, only the highest rate (i.e 170 kgN ha^{-1}) showed a significant higher N accumulation whereas, all the other treatments performed similar to the control. On the contrary, ANR was not affected by the fertilization rate. However, the mean final ANR in this study (i.e. 24.7 %) is higher than the final ANR found by Drakopoulos (2014) and Rodriguez (1996) under solid cattle manure application (respectively 18% and 10.3%). The other crop performance indicator taken into account was Partial Factor Productivity (PFP). For this indicator, no significantly difference was found in both factors. However, PFP under C : N ratio 16 treatment (i.e. 115 kg yield kg $Napplied^{-1}$) is two or three times higher than previous experiment on solid cattle manure (39 and 25 kg yield kg $Napplied^{-1}$) and lucerne pellets (59 and 39 kg yield kg $Napplied^{-1}$) (Drakopoulos, 2014; Terra, 2015).

Table 9. C : N ratio (24, 22, 17, 16) and fertilization rate (control, 57, 113, 170 kgN ha^{-1}) effect on aboveground, tuber and total nitrogen accumulation (kgN ha^{-1}) at 10, 14 and 20 WAP (Final harvest). Different letters refer to significant differences according to Fisher's protected LSD-test (P<0.05).

	N accumulation aboveground (kgN ha^{-1})		**N accumulation tuber (kgN ha^{-1})**			**N accumulation total (kgN ha^{-1})**	
	10 WAP[1]	14 WAP	10 WAP	14 WAP	Final harvest	10 WAP	14WAP
C : N Ratio (F)							
24	75 c	50.1	5 b	127	112	80 b	177
22	87 bc	50.0	13 a	105	118	99 a	155
17	96 ab	50.5	13 a	110	119	108 a	160
16	102 a	51.7	10 ab	113	131	111 a	165
Significance[2]	**	ns	*	ns	ns	**	ns
Fertilization rate (R)							
0 (Control)	50 c	25 c	8	67 b	90 b	58 c	91 c
57	79 b	37 c	8	86 b	102 b	87 b	123 c
113	92 ab	51 b	12	116 a	113 b	103 a	167 b
170	100 a	64 a	11	139 a	145 a	109 a	203 a
Significance	***	***	ns	***	***	***	***
F x R	ns	ns	ns	ns	ns	ns	ns

[1] WAP= Weeks after planting

[2] *, ** and *** refer to *P* values < 0.05, < 0.01 and < 0.001, respectively; ns = not significant.

Table 10. C : N ratio (24, 22, 17, 16) and fertilization rate (control, 57, 113, 170 kgN ha^{-1}) effect on Apparent Nitrogen Recovery (ANR) (%) at 10, 14 and 20 WAP (Final harvest), N uptake (kgN ha^{-1}) between 0-10 and 10-14 WAP and Partial Factor Productivity (PFP) (kg yield kg Napplied ha^{-1}). Different letters refer to significant differences according to Fisher's protected LSD-test (P<0.05).

	ANR (%)			**N uptake (kgN ha^{-1})**		**PFP (kg yield kg Napplied^{-1})**
	10 WAP[1]	14WAP	Final harvest	0 - 10 WAP	10 - 14 WAP	Final
C : N Ratio (F)						
24	16 b	68	13	80 b	98 a	80
22	42 a	64	26	99 a	55 b	74
17	50 a	55	26	108 a	54 b	108
16	53 a	65	33	111 a	52 b	115
Significance[2]	**	ns	ns	**	*	ns
Fertilization rate (R)						
0 (Control)	-	-	-	58 c	33 b	-
57	50	56	21	87 b	36 b	94
113	40	67	21	103 a	64 ab	89
170	30	66	33	109 a	94 a	100
Significance	ns	ns	ns	***	**	ns
F x R	ns	ns	ns	ns	ns	ns

[1] WAP= Weeks after planting

[2] *, ** and *** refer to *P* values < 0.05, < 0.01 and < 0.001, respectively; ns = not significant.

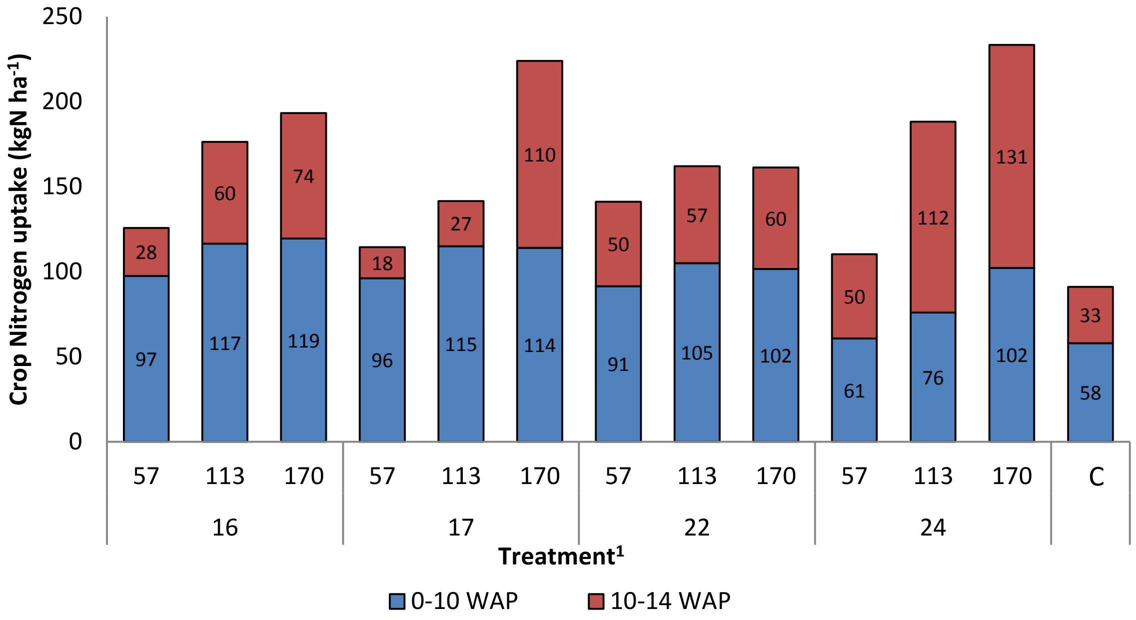

Figure 6. Crop Nitrogen uptake (kgN ha^{-1}) influenced by Fertilization rate (0 Control (C), 57, 113, 170 kgN ha^{-1}) and C : N ratio (16, 17, 22, 24) between 0-10 and 10-14 WAP.

[1]Top line of treatment refers to fertilization rate, bottom line refers to C : N ratio

6.5 Soil N, P, K content

Available mineral nitrogen (N_{min}) content at 0-30 cm soil layer was not influenced by the C : N ratio of the silage applied in all soil sample collections (Table 11). On the contrary, fertilization rate influenced significantly N_{min} in all soil samplings. At 7 and 13 WAP, only the highest rate (i.e. 170 kgN ha^{-1}) was higher than the other fertilization rates. At 20 WAP, the two highest fertilization rates (i.e. 113 and 170 kgN ha^{-1}) had the highest N_{min} (Table 11). At 20WAP, N_{min} in the control (29 kgN ha^{-1}) was 8 times higher than the initial value (3.6 kgN ha^{-1}). The source might be the N mineralized from the mixture of mustard and radish cover crop grew during the winter. In fact, Mustard is an efficient catch crop able to uptake 34 to 51% of the N available in the soil (Collins, 2007).

P content a 0-30 cm soil layer was not influenced by both C : N ratio and fertilization rate factors showing homogenous values at every samples collection (Table 11). Although P content in the silage was rather low (i.e. 0.3%, data not shown), P content in the above ground material and tubers was adequate in all the treatments, control included (Table 8).

At 7 WAP, available K at 0 – 30 cm soil layer was not influenced by C : N ratio or fertilizer rate (Table 11). Due to the unexpectedly high K content in the samples (more than 300 kgK ha^{-1}), it is likely to consider a contamination during the extraction. At 13 and 20 WAP available K was highly influenced by the C : N ratio of the silage. In the first case, the two highest C : N ratios (i.e. 24 and 22) shown significantly higher values compared to the two other treatments (Table 11). On the contrary, at 20 WAP, C : N ratios 16 and 17 show higher available K compared to the other treatments. At 13 and 20 WAP, available K in the soil was also highly influenced by the fertilization rate (Table 11). 170 kgN ha^{-1} and the control show respectively the highest and the lowest value in both soil samplings. At 13 WAP, 57 and 113 kgN ha^{-1} application rates gave intermediate results. Finally at 20 WAP, 57 kgN ha^{-1} application rate performed as the control (Table 11).

Table 11. C : N ratio (24, 22, 17, 16) and fertilization rate (0 (Control), 57, 113, 170 kgN ha^{-1}) effect on available mineral nitrogen (N_{min}), P-PO_4 and K (kg ha^{-1}) in 0 - 30 cm soil layer at 7, 13 and 20 WAP. Different letters refer to significant differences according to Fisher's protected LSD-test (P<0.05).

	N_{min} 0-30 cm soil layer (kgN ha^{-1})			**P-PO_4 0-30 cm soil layer (kgP ha^{-1})**			**K 0-30 cm soil layer (kgK ha^{-1})**		
	7 WAP[1]	13 WAP	20 WAP	7 WAP	13 WAP	20 WAP	7 WAP	13 WAP	20 WAP
C : N Ratio (F)									
24	61	21	33	4.4	3.5	4.9	361	216 a	143 c
22	68	22	39	4.0	3.7	5.0	370	197 a	157 bc
17	71	21	37	4.0	3.6	5.4	321	149 b	200 a
16	80	24	36	4.3	4	4.9	326	146 b	185 ab
Significance[2]	ns	ns	ns	ns	ns	ns	ns	***	**
Fertilization rate (R)									
0 (Control)	57 b	23 ab	29 c	4.4	3.9	5.1	192	107 c	120 c
57	65 b	20 b	32 bc	4.3	3.6	4.7	370	150 bc	135 c
113	65 b	20 b	37 ab	4	3.7	5.0	324	173 b	172 b
170	79 a	26 a	40 a	4.3	3.7	5.4	334	210 a	207 a
Significance	*	**	**	ns	ns	ns	ns	***	***
F x R	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1] WAP= Weeks after planting

[2] *, ** and *** refer to *P* values < 0.05, < 0.01 and < 0.001, respectively; ns = not significant.

6.6 Silage N,P,K release

As shown in figure 7, the four C : N ratio treatments affected N release. N released from C : N ratio 24, 22 and 17 did not differ during the first two weeks (approximately 40% in all the treatments). On the other hand, C : N ratio 16 released N significantly faster (i.e. 60% after 2 weeks). At 14 weeks after burying (WAB) all the treatments released 80% circa of the total N. Hence, to confirm our fourth hypothesis (IV), C : N ratio 16 led to a faster initial N release compared to the other ratios. This faster release is not visible in crop performance. In fact, at 2 WAB potato plantlets were not emerged yet. However, further specific research is needed to find out the effect of C : N ratio in green manure on N losses. A study carried out by Carter *et al*. (2014) showed a relatively high Nitrous oxide (N_2O) volatilization (0.3% N_2O volatilized of N applied) by grass-clover amendment (C : N ratio 19.5) incorporated by ploughing. This is relevant because N_2O is the most concerning greenhouse gas in terms of ozone layer reduction (IPCC, 2013).

P release was influenced by the C : N ratio of green manure (Fig. 8). P release was slower increasing C : N ratio of the silage. At 2 WAB, C : N ratio 16 released 70% whereas the other three treatments released 50% circa of total P. At 14 WAB, C : N ratio 16 released 90% of total P, whereas the other treatments released between 70 and 80%.

K release was influenced by the C : N ratio during the first 2 weeks. C : N ratios 24 and 22 show a slower release (respectively 65 and 80%) whereas 17 and 16 was faster (90 and 95%) (Fig. 9). However, at 14 WAB, all the treatments released approximately 92 - 97% of total K. Therefore, it appears that grass-clover fertilizer releases K relatively fast.

Similar results were found by Palm *et al.* (1991) on N, P, K release by the leguminous species E*rythrina* sp. Their results show a N, P, K release of 80, 55 and 90% respectively, which is comparable to our findings. However, they argue that this fast nutrients release was affected mostly by low lignin : N ratio and low polyphenols content rather than low C : N ratio. These two factors were not taken into account in this study but it might be a starting point for further research.

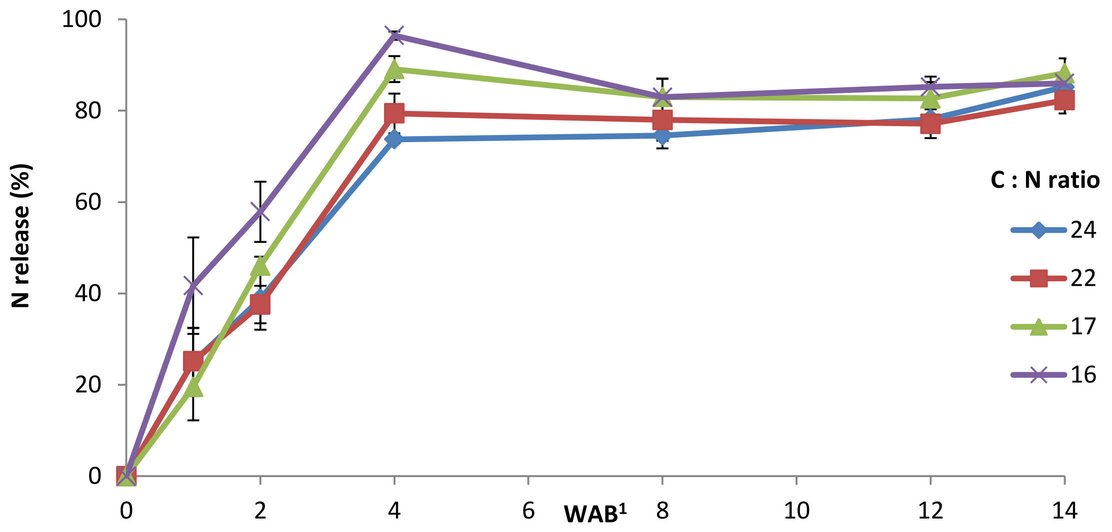

Figure 7. Nitrogen release (%) between 0 – 14 weeks after burying (WAB) from different C : N ratio grass-clover silages (24, 22, 17, 16). Error bars represent standard error.

[1] WAB = Weeks after burying

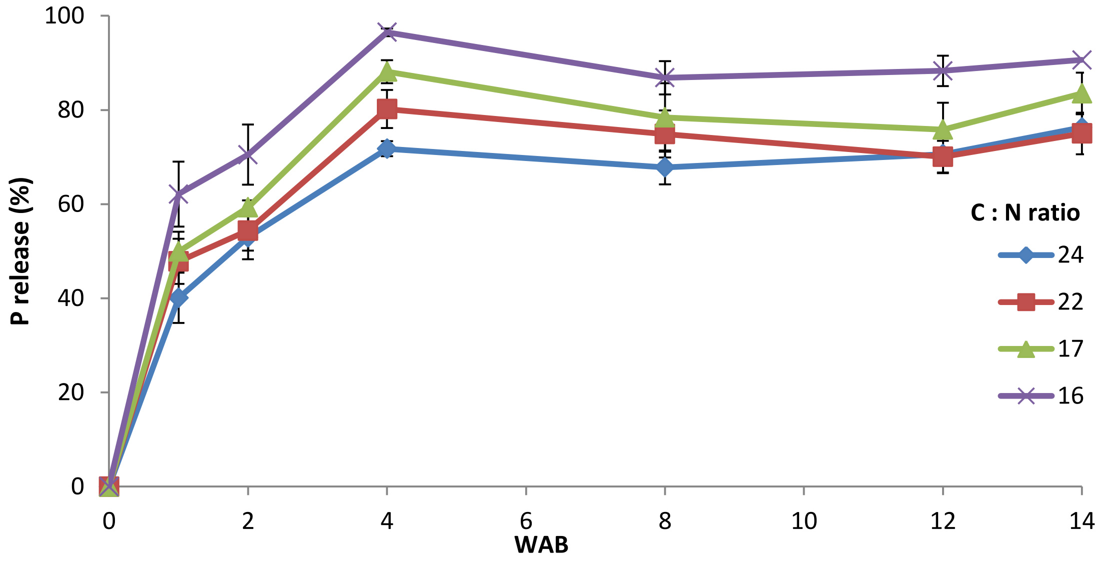

Figure 8. Phosphorus release (%) between 0 – 14 weeks after burying (WAB) from different C : N ratio grass-clover silages (24, 22, 17, 16). Error bars represent standard error.

[1] WAB = Weeks after burying

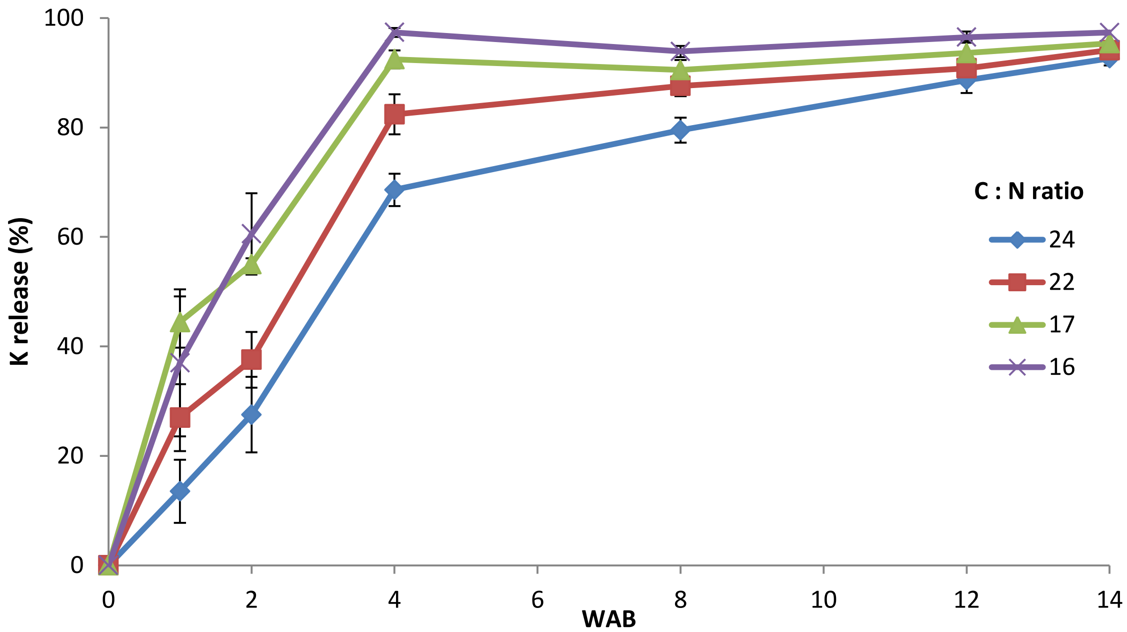

Figure 9. Potassium release (%) between 0 – 14 weeks after burying (WAB) from different C : N ratio grass-clover silages (24, 22, 17, 16). Error bars represent standard error.

[1] WAB = Weeks after burying

7. Conclusions

The aim of the study was to investigate the effect of different C : N ratios in grass-clover fertilizer on fresh tuber yield, tuber quality, and N, P, K content in the plant. Here we demonstrated that C : N ratio has an influence on the fresh tuber yield. C : N ratio 16 led to a slightly higher yield (i.e. 43.6 ton ha^{-1}) compared to C : N ratios 24 and 22 (i.e. 39.9 and 39.3 ton ha^{-1} respectively). High C : N ratio had a significant negative effect on N accumulation. At 10 weeks after planting, total N accumulation by C : N ratio 24 was lower compared to the other treatments (i.e. 80 and 105 ± 6 kgN ha^{-1} respectively). On the contrary, the C : N ratio of the silage did not influence the starch content and dry matter of the tubers. Likewise, ANR , PFP and N, P, K content in potato plants were not influenced by the C : N ratio. The mean ANR and PFP shown in all four treatments (i.e. 24.7 % and 94 kg yield kg $Napplied^{-1}$ respectively) were higher than previous experiments on solid cattle manure.

Furthermore, this study explored the effect of N fertilization rate on fresh tuber yield and tuber quality. N fertilization rate had a significant influence on fresh tuber yield. Every increase in fertilization rate by 57 kgN ha^{-1} increased the tuber yield by 5-7 ton ha^{-1}. Hence, the highest fertilization rate performed the best (i.e. 47.5 ton ha^{-1}) with an increase in yield higher than 50% compared to the control (i.e. 30.5 ton ha^{-1}). On the contrary, higher N application rate influenced negatively tuber quality in terms of tuber DM (%) and starch content (%) whereas the control performed the best. In both, the difference between the highest (20.4 % DM and 13.6 % starch of the control) and the lowest value (i.e. 19 % DM and 12.9 % starch of 170 kgN ha^{-1} treatment) was relatively small. On the contrary, fertilization rate had a positive impact on the large tuber category. An application rate of 170 kgN ha^{-1} had almost twofold large tubers yield (i.e. 39 ton ha^{-1}) compared to the control (i.e. 21 ton ha^{-1}).

Finally, we studied the effect of C : N ratio in grass-clover silage on its N, P, K release. Grass-clover silage mown in October-November (C : N ratio 16) shown an initial faster N release (i.e. 60% N released after 2 weeks) compared to the other treatments (i.e. 40% circa) . After 14 weeks, N release was approximately 80% in every treatment. Likewise, both P and K initial release were affected by C : N ratio of the silage, whereas lower C : N ratio released P and K faster. However, it might be interesting to explore the influence of other silage quality characteristics. In fact, in this study, some aspects of silage (i.e. lignin : N ratio, polyphenols) were not considered and they might be a starting point for further research.

To conclude, grass-clover cut-and-carry fertilizer is a potential substitute of animal manure in the Netherlands. This study confirms its effectiveness in agriculture in terms of nutrient utilization by the crop (ANE and PFP) and crop yield (tuber yield).

8. References

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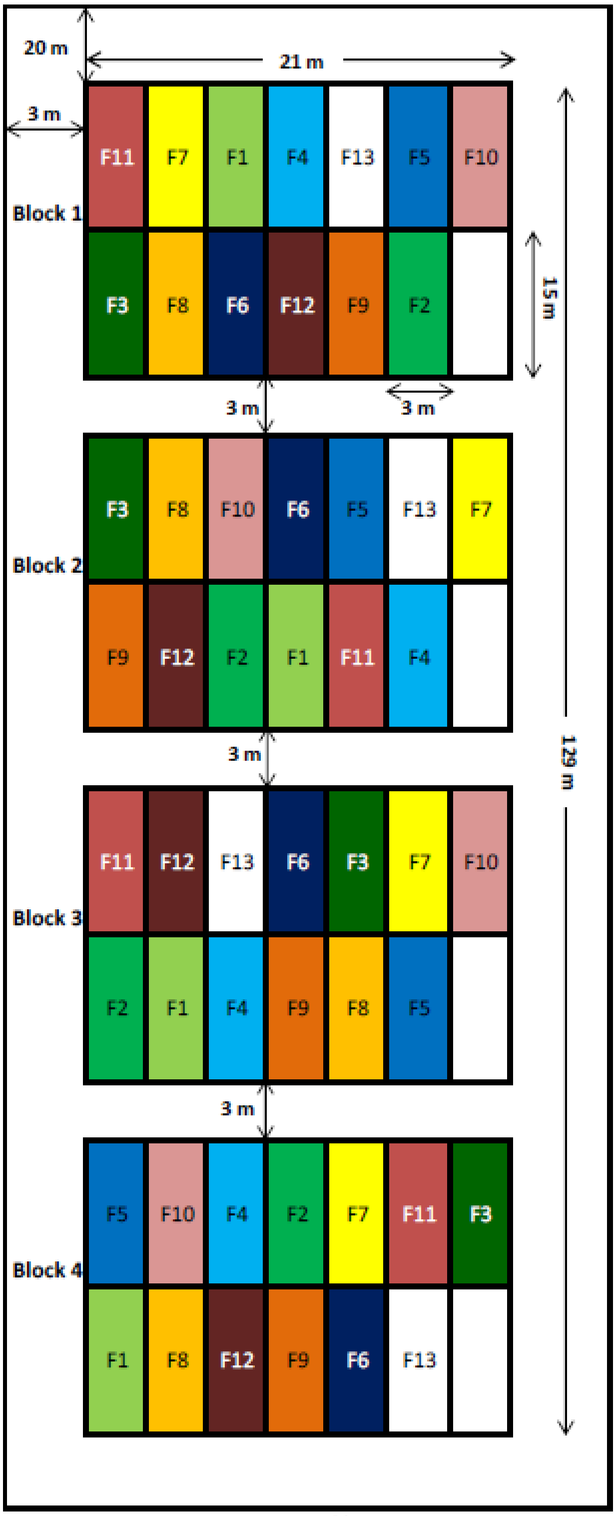
9. Appendix



Experimental layout

Legend

Treatment # (Field #)	C:N ratio	Application rate (kg N ha^{-1})
1	24	57
2	24	113
3	24	170
4	22	57
5	22	113
6	22	170
7	17	57
8	17	113
9	17	170
10	16	57
11	16	113
12	16	170
13	C[1]	0

[1] Control

Figure 10. Experimental layout.

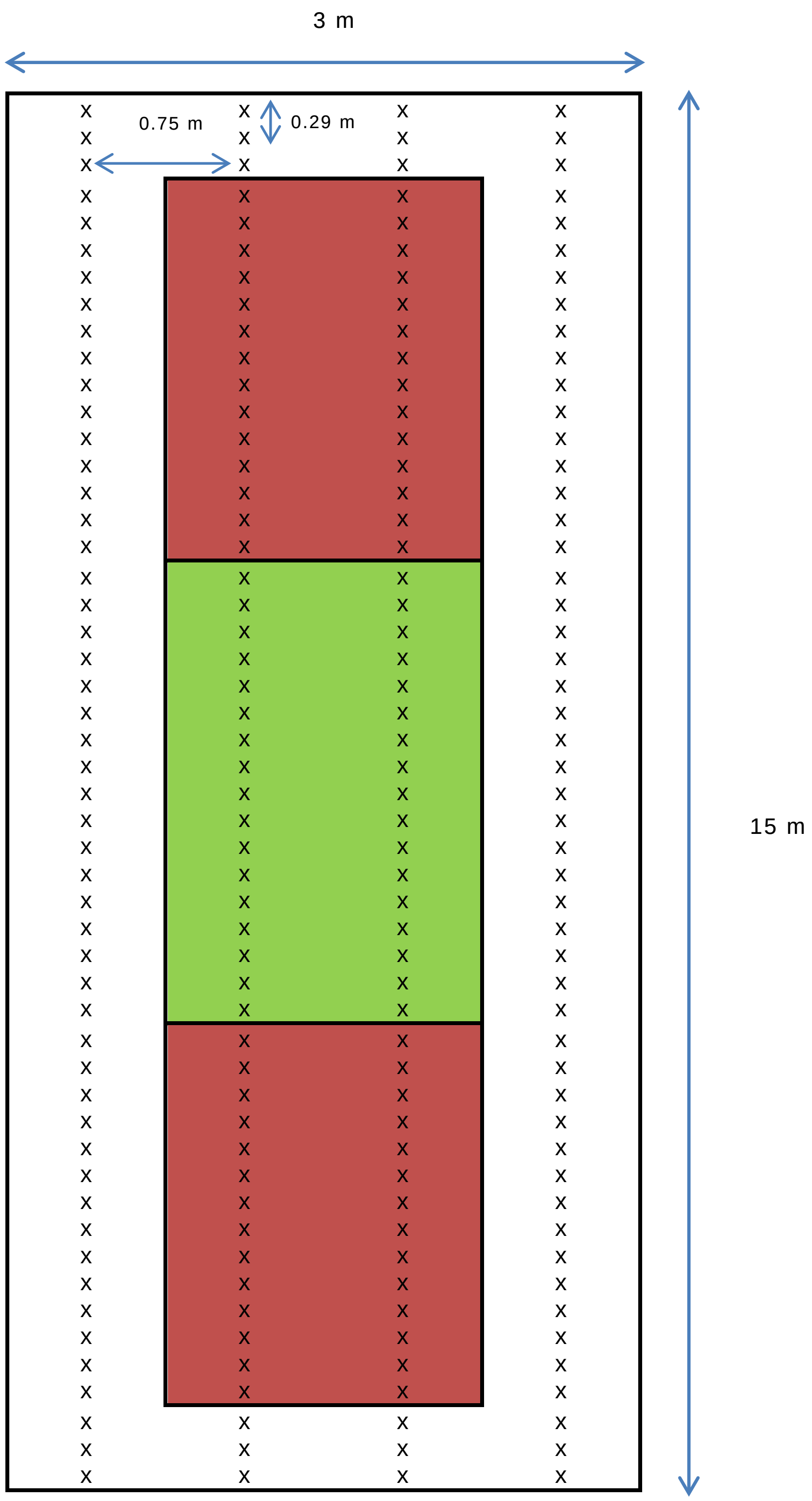


Figure 11. Plot layout. Every x is a potato plant. The external part of the plot was not measured to avoid border effects. Red and green area form the net plot. Plants in the red area were harvested for pre-harvest destructive measurements. Plants in green area were harvested for the final harvest.