Influence of grass-clover silage quality and application rate on crop performance in organic potato production





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Preface

This study investigated the effects of C: N ratio (range 16-24) of grass-clover silage and nitrogen application rate (57, 113 and 170 kg N ha⁻¹) on above ground crop performance, tuber yield and agronomic nitrogen efficiency in organic potato production. This research was carried out at Droevendaal organic research facility of Wageningen in The Netherlands as part of my MSc Programme in Plant Sciences (Natural Resource Management) at Wageningen University. The opportunity I got to conduct my research at the Droevendaal farm under the close supervision of Egbert Lantinga of the Farming Systems Ecology (FSE) group left me with a great knowledge and experience in applied field research. This has contributed to increasing my insight in scientific research and might help me for my future academic career. The knowledge and experience gained during this thesis will also contribute to the scientific community.

This thesis would not have been possible without the supervision of Egbert Lantinga and I would like to extend my gratitude to you for your dedicated supervision. Also, I would like to thank the group of students (Pauline Martel and Ivan Palomba) for their collaboration and support during my MSc thesis. Special thanks go out to Andries Siepel and other staff members of Unifarm for the practical work and Hennie Halm for the laboratory analysis. Eventually, I would like to thank my friends for supporting me in finishing my MSc thesis.

Abstract

Potato (Solanum tuberosum L.) is one of the major crops contributing to the world's food requirement. It is one of the most important field crops contributing to the food which is not only used for local consumption but also it helps to increase income through its exportation. Many studies have been carried out on the growth, productivity and nutritional properties of potato, including the effect of organic manures on above-ground crop performance, tuber yield, and quality. However, there exists no study on the effect of grass-clover silage quality (C: N ratio) and its application rate on organic potato production. The main objective of this study was to investigate the effect of C: N ratio (16, 17, 22, and 24) and nitrogen application rate (57, 113 and 170 kg N ha⁻¹) on above-ground crop performance, tuber yield and agronomic nitrogen efficiency (ANE; kg tubers per kg N applied) in organic potato production. There was also a non-amended control treatment where no N was being applied. In total, there were thirteen different treatments and each treatment was replicated four times using a randomized complete block design. The study was carried out on a silty sand soil in Wageningen, The Netherlands. There were interactive effects between C: N ratio and N application rate on above-ground plant dry weight and leaf area index (LAI) at 14 weeks after planting (WAP). From 9 up to 13 WAP leaf chlorophyll content for the highest C: N ratio (24) was lower than the other C: N ratios probably due to immobilization during initial crop growth. Although total tuber yield showed no big difference between the different C: N ratios, it increased linearly with increasing N application rate and was higher compared to the potato tuber yield of the previous studies. In addition, above ground fresh weight, above ground dry weight, leaf area index, marketable yield and large tubers (> 40 mm) all increased significantly with decreasing C: N ratio and increasing N application rates. However, plant emergence, agronomic nitrogen efficiency and cull tubers (small and damaged tubers) were not affected by silage C: N ratio as well as N application rate. On the other hand, the low N application rate (i.e., 57 kg N ha⁻¹) increased the fraction of small tubers (i.e., 15-40 mm) but had a positive effect on tuber quality (i.e., specific gravity and starch content). Leaf chlorophyll content appeared to be the best predictor for the final tuber yield and at 13 WAP it accounted for about 80% of the final yield variability. This study shows that the treatments with lowest C: N ratio (16) and highest N application rate (170 kg N ha⁻¹) performed better regarding leaf chlorophyll content, above ground fresh weight, above ground dry weight, leaf area index, fresh tuber yield and the fraction of large tubers. However, there were no significant differences with respect to ANE, plant emergence, the percentage of culls and only a very small negative but significant effect on tuber quality (specific gravity and starch content) was observed. Farmers can use pasture management of grass-clover in order to get the clover with high N content to reduce the risk of initial N immobilization.

Keywords: Organic potato production, C: N ratio, nitrogen application rate, above ground crop performance, potato tuber yield, agronomic nitrogen efficiency.

1. Introduction

1.1 Organic agriculture in the Netherlands

The organic farming sector in the Netherlands is small compared to most other European countries. In 2012, the share of organic farming in the total Dutch farmland was almost 3% whereas for instance Austria shared 19%, Sweden 15%, Estonia 15% and the Czech Republic 13% (Eurostat, 2014). In 2014, about 60% of this area consisted of dairy farms (CBS, 2015). At the end of the 1990s, there was a relatively strong increase in the organic land area but this was not continued after 2004. However, in 2010 and 2011 there was again a significant increase in the organic land area but stabilized thereafter (CBS, 2015). The provinces that have the largest share of total area of organic farmland in the Netherlands are Flevoland (17%), Gelderland (13%) and Friesland (12%) whereas the lowest share is in Limburg (2%), Zeeland (3%), South Holland and Utrecht (both 5%). In the other 5 provinces, the share is between 6 and 10% (CBS, 2015).

1.2 Organic potato production

Potato (*Solanum tuberosum L*.) is one of the major crops contributing to the world's food requirement (Karam et al., 2009). Potato occupies the fourth place in the global production after maize, rice, and wheat (Stephen and Jackson, 1999; Bower et al., 2003). From 2007 to 2008, the Netherlands accounted 1,271 ha of the area under organic potato production, 2.5% of organic potatoes in total organic production and less than 1% of organic potatoes in total potato production (Canali et al., 2012).

The yield per unit area and total production in organic production will be reduced due to the constraints of crop protection and nutrient management. Late blight caused by a fungus such as an oomycete pathogen, *Phytophthora infestans*, lowers potato yield while their control by using chemical fungicide is not allowed in organic production systems (Kuepper et al., 2001). Another factor that prevents potato production is the fact that, as a high mining crop, it requires a higher amount of NPK elements for its economic tuber production (Bishnu and Krisma, 2006).

Under organic production, land productivity could be improved by using of organic fertilizer in order to provide adequate nutrients in the soils (Souchez et al., 1997). However, the decomposition rate depends on organic materials and nutrient release capacity. In Dutch organic agriculture, the other way for enhancing both soil and soil quality without dependent of external animal fertilizer is the use of cut-and-carry fertilizers (Scholberg et al., 2009).

1.3 Use of cut-and-carry fertilizers

In Europe organic farming systems, grass-clover pasture is generally used as feed for livestock but recently also as green manure fertilizer for in agriculture production. The N production from grass-clover varies depending on growing conditions and species. Thus, N production per year ranging from about 300 kg N ha⁻¹ in grass-clover white clover and 400 kg N ha⁻¹ in alfalfa or grass-clover with red clover (Rasmussen et al., 2012). The contribution of grass-clover for N supply to the crop depends considerably on the composition of the plant material, time of material incorporation with climatic conditions association (Askegaard and Eriksen, 2007; Ball et al., 2007).

During the season, the application of fertilizer is required to ensure high yield and quality in organic crop production. Grass-clovers as a source of nutrients are not only important for increasing yield but also for providing phosphorus and potassium to the crops without risk of leaching and volatilization. In addition, the plant-based fertilizers can be used to avoid the risk of contamination of edible plant products with the slurry (Sorensen and Thorup-Kristensen, 2011). The green manures used as fertilizers come from leguminous crops, and their incorporation into the soil provides nitrogen and carbon which are important in organic crop production (Cherr et al. 2006). The fertilizer has to be the rich and fast release of nutrients. In addition, the fertilizer has to keep well and its transport, application and soil incorporation must be easy. Mobile green manure refers to the green manure material that is harvested, stored, and applied as a fertilizer. Therefore, it is different to the traditional green manure crop that is ploughed in completely (Sorensen and Thorup-Kristensen, 2011).

Results from different studies stated that green manures with high nutrient concentration and low C: N ratio used as fertilizer can significantly increase yield in organic crop production systems (Niemsdorff and Muller 2006; Thorup-Kristensen 2006; Elfstrand et al. 2007; Sorensen and Thorup Kristensen 2011). Green manure can be applied as fresh or dried material. The fresh material is not easy to handle during application and nutrients are not available immediately to the crop but can be released as the material is being decomposed (Sorensen and Thorup-Kristensen, 2011). The decomposition of plant material is more influenced by soil humidity and temperature but also N, P, and S concentration in relation to carbon concentration and to the concentration of resistant constituents such as cellulose and lignin (Müller and von Fragstein Und Niemsdorff, 2006; Ha et al., 2008). In the previous study, the vegetable (e.g. kale) production decreased when the carbon-tonitrogen (C: N) ratio of applied green manure increased from 10 to 20 and the differences in vegetable production were not due to the amount of N applied, but to the N availability (Sorensen and Thorup-Kristensen 2011). This is due to low N release of plant residues by the microorganisms. In this case, the microorganisms (fungi and bacteria) assimilate the available nutrients especially N for their own growth and multiplication before they make it available to the crops in mineral form. The C: N ratio has to be low with high N release to get a fast response (Sorensen and Thorup-Kristensen 2011).

The level of N input has no effect on CO₂ assimilation. This could be explained by a down-regulation in the relationship between leaf N concentration and maximum leaf CO₂ assimilation rate from low to high N input levels. It may be attributed to high leaf N which is not present in active Rubisco but in inactive Rubisco form, amino acids, amides and inorganic N at high N fertilization (Lantinga et al., 1996). However, the effects of N fertilization are much larger for leaf area increase than for the level of CO₂ assimilation per unit leaf area. Increasing production of all biochemical components would increase biomass and demand for nitrogen and maintain C: N ratio (Lawlor, 2001). Nitrogen deficiency has a great impact on chloroplast with low size, composition, and function (Laza et al., 1993). Furthermore, studies showed that intensified nitrogen fertilization decreased the content of dry matter and starch in potato tuber (Leszczyński and Lisińska, 1988).

1.4 Purpose of the study

1.4.1 Problem statement

One of the major concerns in today's words is the pollution and contamination of soil due to the use of excess chemical fertilizers and pesticides (Tabatabai et al., 2014). The use of solid cattle manure in organic agriculture was found the best way of increasing yield because it contains N in a mineral form which can be used immediately by the crop during initial crop growth (Olesen et al., 2009). Other research demonstrated that the use of cattle slurry can contribute to the transmission of the pathogen on edible plant products and plant- based fertilizers can be used to avoid this risk (Sorensen and Thorup-Kristensen 2011). The cut-and-curry fertilizer can reduce this problem by providing the nutrients matching closer with the crop demand which minimizes also the risk of leaching and volatilization. Despite this role that cut-and-carry fertilizer can replace the cattle slurry for maintaining and improving soil fertility in organic agriculture production, the cut-and-carry fertilizer is not extensively used in organic agriculture production. There is a lack of information on the best quality of grass-clover silage (C: N ratio) and its application rate for the good performance of above-ground crop growth, tuber yield and agronomic nitrogen efficiency in organic potato production. The last study focused on the comparison between the use of glass-clover and other sources of nitrogen in organic potato production (Terra, 2014).

1.4.2 Research aim

The main objective of this study was to investigate the effect of C: N ratio (16, 17, 22, and 24) and nitrogen application rate (57, 113 and 170 kg N ha⁻¹) on above ground crop performance, tuber yield and agronomic nitrogen efficiency (ANE; kg tubers per kg N applied) in organic potato production. In specific, this study was conducted to investigate the best C: N ratio and application rate in organic potato production for better above ground crop performance, potato yield and agronomic nitrogen efficiency. In addition, the effect of C: N ratio and its application rate on tuber quality (specific gravity and starch content) were determined. Thus, these provide more insight on the effect and use of grass-clover silage in organic potato production and intensify the utilization of cut-and-carry fertilizer in organic farming.

1.4.3 Research questions

-What is the best grass-clover silage quality (C: N ratio) and application rate for above ground crop performance, tuber yield and agronomic nitrogen efficiency in organic potato production?

-Is there any effect of the C: N ratio and its application rate on tuber quality (specific gravity and starch content)?

1.4.4 Hypotheses

- 1. The use of grass-clover silage with a low C: N ratio of 16 will result in better above ground crop performance, tuber yield, and agronomic nitrogen efficiency because it releases early and more nitrogen during decomposition compared to silage with high C: N ratio.
- 2. High nitrogen application rate of 170 kg N ha⁻¹ will show better above-ground crop performance and tuber yield and will have a negative effect on agronomic nitrogen efficiency compared to low N application rate.

- 3. The treatments with low C: N ratio and high N application rate are expected to give the low value of specific gravity and starch content compared to the treatments with high C: N ratio and low N application rate.
- 4. Leaf area index will be a better predictor for the final yield compared to the leaf chlorophyll content.

1.5 Structure of the thesis

This report consists of five chapters. The first chapter presents the general background information followed by the research aims, research questions, and hypotheses while the second chapter describes the Materials and Methods. The third chapter includes the Results and Discussion section on above-ground plant growth measurements, agronomic nitrogen efficiency, and tuber yield. The conclusions and recommendations are described in the last two chapters (4 and 5).

2. Materials and methods

2.1 Experimental site

The experiment was conducted at the Droevendaal certified organic research and demonstration facility of Wageningen University. This farm has 50 ha and its climate is temperate oceanic with an annual mean temperature of 11°C and annual mean precipitations of 829 mm. The soil type is silty sand with 3% clay (< 2 μ m), 15% silt (2.50 μ m) and 82% sand (> 50 μ m). Organic crop rotation on the farm has been practised for 12 years, including winter triticale, grass-clover, and spring wheat with field beans, catch crops, and potatoes. The previous crop was summer barley.

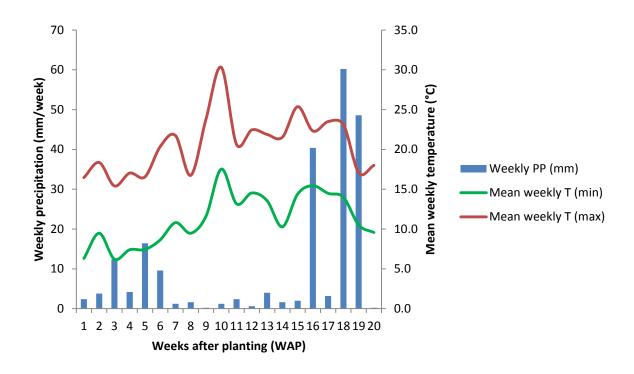


Fig. 1 Weekly precipitation and temperature during the potato production period (4/22/2015 - 9/10/2015).

2.2 Experimental design

The experiment was designed as a Randomized Complete Block Design (RCBD) with 4 replicates. Two factors such as C: N ratio and the nitrogen application rate are contained in the experiment. The former factor had 4 levels while the latter had 3 levels (Table1). Including the control, the experiment had 13 treatments, which results in 52 plots (Figure 2). There was the supplemental control plot (F14) which was used for the other measurements. The experimental unit is a plot with 45 m² (15 m × 3 m), with four rows of potatoes.

Table 1. Factors and levels used in the experiment

Factors	Levels
C : N ratio	Control/ No fertilization, 16, 17, 22, 24
Application rate (Kg N ha ⁻¹)	0/control, 57, 113, 170

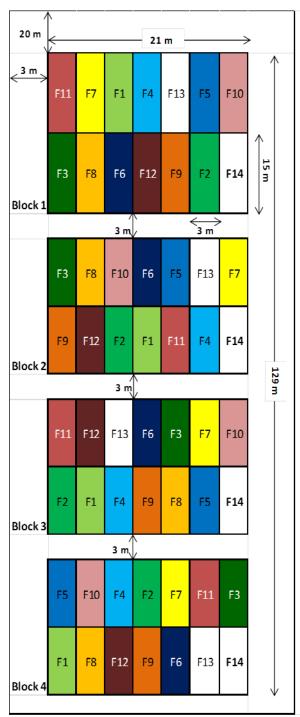


Table 2. Treatment list (Legend)

		A 12	
Treatment #	C:N ratio	Application rate	
(Field #)	CINTALIO	(kg N ha⁻¹)	
14	-	0	
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	-	0	

Fig. 2 Experimental layout

2.3 Agronomic practices

In 2014, the grass-clover fields were cut four times. After mowing they dried in the field for one to three days where after they were compressed in round bales and covered in plastic film to be ensiled during winter. Before planting, the grass-clover silages were chopped and spread by hand in the way that there was an equal distribution with 57, 113, and 170 kg N ha⁻¹. The incorporation of grass-clover silages into the soil was done by using ecoplough to a depth of 15 cm. The potato variety used was Carolus. This variety is organic, late and resistant to *Phytophthora infestans* (Agrico, 2015). The planting time was on April 22, 2015, with an inter-row spacing of 0.75 m and an intra-row spacing of 0.29 m (Agrico, 2015) with the use of GPS. The ridges were formed at the same time during planting and re-ridging was done once during the growing season on May 20, 2015. Additional weeding was done by hand. No chemical pest control was used; the monitoring of the crop was done especially during the vegetative growth stage of the crop. Manual removal of some *Colorado beetles* was done where they were presented on the crops.

2.4 Field measurements and laboratory analysis

2.4.1 Pre-experimental analysis

Determination of chemical composition of grass-clover silage

To ensure the grass-clover silages with good C: N ratio to use during experimentation, the grassclover silages were analysed on 11th March 2015. Dry matter, organic matter, ash content, total carbon and Nitrogen content were determined in order to get the C: N ratio of each of the silages.

Initial soil analysis

Before applying the fertilizer, soil analysis was done in order to determine the initial soil status. Per each block, 10 samples were collected from a 0-30 cm soil depth according to a zig-zag pattern by using of soil auger. After that, the soils from every block were mixed in order to form a single sample. In every sample, the sub-sample was taken and analysed in order to determine SOM%, N, P, K, and pH.

2.4.2 Crop performance measurements

In order to get viable measurements without border effects, the net plot was delimited within plot for measurements (Appendix 1). In four rows per plot, two internal rows were used for measurements. This means that two external rows and 3 first and 3 last plants of the two internal rows were not used for measurements. For destructive measurements, 6 plants located behind the 3 first plants and before the 3 last plants in two internal rows were used. The net plot had two inside rows where the plants were spacing with 0.75 m in inter-rows and 0.29 m in intra-row.

Plant emergence

Plant emergence day after planting was measured within the net plot. The number of plants emerged was counted in the interval of 2 days from the first day that plant emergence was observed. The day of emergence was determined while more than or equal 50% of the plants emerged. The following formula was used to calculate the plants emergence date (ED) in the day after planting (DAP).

ED (DAP) = T1+ (T2-T1)
$$\times \frac{Target-M1}{M2-M1}$$
 (Drakopoulos et al. 2014)

Where:

T1 = The days after planting (DAP) when < 50% have emerged

T2 = The days after planting (DAP) when \geq 50% have emerged

Target = 50% of emerged plants

M1 = The plant number emerged measured at T1

M2 = The plant number emerged measured at T2

Leaf area index (LAI), Above ground plant fresh weight and Above ground plant dry weight

The LAI, above ground plant fresh weight and above ground plant dry weight measurements, were determined at 8, 10, and 14 weeks after planting (WAP). Two plants were harvested per plot. These two plants were selected randomly from the 6 plants situated behind the 3 first plants and before the 3 last plants in two internal rows.

Leaf area index refers to the total leaf area of the plant divided by the area of the soil covered by the plant. The leaf area was measured by using leaf area meter (LI3100, Licor, Lincorn, NE, USA). We took the leaf area of two plants and divided the area covered by two plants in order to calculate the LAI (m2/m2).

Leaf area index (LAI) = A_{leaf}/A_{soil}

A_{leaf}: Leaf area (m²)

A_{soil} : Ground area (m²)

The above ground plant fresh weight is the fresh weight of above ground plant parts. This measurement used the same plants of LAI measurement. After measuring, the above ground plant fresh weight of two plants was divided by two in order to calculate the amount of above-ground plant fresh weight for one plant. The above ground plant fresh weight was expressed in ton/ha.

The above ground plant dry weight is the dry weight of the total above ground plant parts. After measurements of above ground plant fresh weight and LAI, the above ground plant parts were dried at 105 $^{\circ}$ C for 24 hours. After drying, the weight of above ground plant parts was measured, and divided by two for determination the above ground plant dry weight of one plant. The unity of expression the above ground dry weight was ton/ha.

Leaf chlorophyll content

The Leaf chlorophyll content was measured at 7, 9, 11, and 13 WAP using a small hand-held chlorophyll meter called SPAD-index meter (SPAD-502, Konica Minolta, Inc, Osaka, Japan) as described by Markwell et al. (1995). In this measurement, the leaf greenness was determined as it is related to leaf chlorophyll content. The fourth leaf counted from the shoot tip was used as it was

considered to be the most recently mature leaf. Four readings per leaf were done on six plants per plot to determine the average of chlorophyll content per plant.

2.4.3 Potato yield measurements

Final yield

The potatoes were manually harvested on September 10, 2015. The two inner rows within each plot of 8.43 m² (5.62 m \times 1.5 m) were harvested. The fresh tuber weight was measured and expressed as tons per hectare.

Tuber grading

Three different categories were used to grad the tubers from the final yield: Large (i.e., > 40 mm), small (i.e., 15-40 mm) and cull (i.e., smaller, damaged and/or fault tubers). After grading, the fresh weights of each size categories were measured. The two first categories (large and small) were considered as the marketable yield. The unity to express the yield was tons per hectare.

Tuber quality

After tuber grading measurement, the tuber specific gravity (SG) was determined on the basis of a representative subsample of 5 kg. SG = W_{air} / (W_{air} - W_{water}); where W_{air} is the tuber fresh weight in air, W_{water} is the tuber fresh weight in water.

According to Simmond (1997), the starch content of potato tubers was calculated as: Starch content = $-1.39 + 0.196 [1000 \times (\text{specific gravity-1})]$.

Agronomical nitrogen efficiency (ANE)

Agronomic nitrogen efficiency was defined to the crop production per unit of nitrogen input. Jamaati-e-Samarin et al., 2010.

ANE
$$\left(\frac{kg}{kg}\right) = \frac{Yield \operatorname{treatment}(kg) - Yield \operatorname{control}(kg)}{N \operatorname{applied}(kg)}$$

Where:

Yield treatment: Total yield (ton ha⁻¹) for one treatment

Yield control: Total yield (ton ha⁻¹) for the control

N applied: Amount of nitrogen applied (kg N ha⁻¹)

The total yield was used for determination of agronomic nitrogen efficiency.

2.5 Statistical analysis

Data analysis was conducted by using Genstat 17th edition (VSN International Ltd., Hemel Hempstead, UK). Analysis of variance (ANOVA) was used to evaluate main effects and interactions. Mean separation was conducted using Fisher's protected LSD-test at 0.05 probability level. Linear and quadratic regression analysis, R², R² adjusted and significance levels were computed using Genstat.

3. Results and discussion

3.1 Chemical composition of grass-clover silage

The chemical composition of different grass-clover silages is shown in table 3. The grass-clover harvested in May 2014 had the lowest N but highest dry matter content and C: N ratio. From grass-clover harvested between October and November 2014 N was 2.6% and it had the lowest dry matter, carbon, and C: N ratio but with highest ash content. The grass-clovers harvested between June-July and between August-September, 2014 had intermediate C: N ratio of 22 and 17 respectively.

Table J.	Table 5. Chemical composition of grass clover shages							
Silage	Harvest time	% DM	% ash	% OM	% C	% N	C/OM	C/N
1	May	73	9	91	44	1.8	0.48	24
2	June-July	64	9	91	44	2.0	0.48	22
3	August-September	47	11	89	46	2.7	0.52	17
4	October-November	19	22	79	41	2.6	0.52	16

Table 3. Chemical composition of grass-clover silages

3.2 Initial soil measurement

The initial soil measurement is shown in table 4. The SOM (%), Nmin (kg N ha⁻¹), and pH (H₂O) were almost the same in all blocks. Thus, the blocks were homogenous for these measurements. However, P (kg P ha⁻¹) was higher in block 1 compared to the other blocks. The value of K (kg K ha⁻¹) was high in block 2 while block 4 had the lowest value.

Table 4. Initial soil measurement

Block	SOM(%)	Nmin (kg N ha⁻¹)	P (kg P ha⁻¹)	K (kg K ha⁻¹)	рН
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

3.3 Plant emergence and leaf chlorophyll content

Plant emergence was not affected by C: N ratio and N application rate and the interaction between these factors were also insignificant (Table 5). The uniformity of germination can be due to the use of homogenous seed potatoes when the environmental conditions (e.g., soil temperature) are the same in all treatments. The sprout growth of tuber bud of potato is induced by phytohormones (gibberellins) (Sonnewald et al., 2011) and during germination the plant depends on the reserves of the seed tubers even if it provided with N (Headford, 1964). The results clearly showed that C: N ratio (N source) and N application rate has no effect on initial growth since the crop initially depends on internal reserve from seed before uptake of soil nutrients.

Leaf chlorophyll content (i.e., SPAD value) was similar for all C: N ratios and application rates at 7 WAP (Table 5). At 11 and 13 WAP SPAD values were almost the same for C: N ratio 16 and 17 and were higher than the C: N ratio 22 and 24. This can be related to high mineralization of grass-clover silage with C: N ratio 16 and 17. The SPAD reading is used to identify severe N deficiencies in a potato while it cannot be used to detect marginal deficiency. At 9 and 11 WAP, the leaf chlorophyll content was lowest for C: N ratio 24, which indicates that the plant N uptake was lower on this treatment because N concentration in the plant was lower. The C: N ratio of the material applied as fertilizer may be the cause of this effect. In chemical fertilizer, N is available to the plant in the form of inorganic. In grass-clover silage depending on the C: N ratio, N is available in organic and in inorganic form. The organic material with C: N ratio above 20 will temporarily immobilize when the material with C: N ratio below 20 will release N (Whitmore, 2006). During immobilization the microbes compete with the plant to the available soil N. It is evident shown that immobilization occurred as SPAD values tended to be low in the treatment with high C: N ratio (e.g., 24 and 22 > 20) and nonfertilized control. The SPAD values at 11 and 13 WAP increased from low N application to high N application rate. Similar results of Semiha (2009) found that leaf chlorophyll content increases with increasing N rate applied. This result also is consistent with the result of Zebarth et al. (2002). At 13 WAP, there was a decline in leaf chlorophyll content. This result was consistent with leaf N decrease with time by Jarrel and Beverly (1981), Güler and Güzel (1994) and Semiha (2009).

	Plant emergence (DAP)	Leaf chlorophyll o	content		
		7 WAP ¹	9 WAP	11 WAP	13 WAP
C: N ratio (F) ²					
24	38.9	50.8	41.8 a	43.3 a	36.8 a
22	40.0	51.2	43.2 b	44.8 b	36.9 a
17	39.5	50.2	43.0 b	46.5 c	38.2 b
16	39.3	51.6	42.9 b	47.1 c	39.3 b
Significance ³	ns	ns	*	***	**
Application rate (R)				
0	39.2	49.4	42.1	41.6 a	33.0 a
57	39.7	51.7	42.6	43.7 a	35.7 b
113	39.8	51.0	42.8	45.9 b	38.3 c
170	38.7	50.2	42.9	46.7 b	39.4 c
Significance	ns	ns	ns	***	* * *
F×R	ns	ns	ns	ns	ns

Table 5. Effect of C: N ratio (16, 17, 22, and 24) and N application rate (0, 57, 113 and 170 kg N ha⁻¹) on plant emergence (days after planting, DAP: 50% of plants emerged) and leaf chlorophyll content (SPAD value) of potato.

¹ WAP = Week after planting.

² Different letters indicate significant differences according to Fisher's protected LSD-test (P < 0.05). ³ *, ** and *** refer to P values < 0.05, < 0.01 and < 0.001, respectively ; ns = not significant.

3.4 Above ground plant fresh weight, above ground plant dry weight and Leaf Area Index (LAI)

Above ground plant fresh weight was not affected by C: N ratio at 8 and 14 WAP (Table 6). However, at 10 WAP, there was an effect of C: N ratio on above ground plant fresh weight. During this time, probably there was the relation between the plant uptake of soil nutrients and N release from grass-clover silage (C: N ratio). The above ground plant fresh weight was higher on the treatment with low C: N ratio 16 compared to the treatment with high C: N ratio 24 while C: N ratio 17 and 22 had the intermediate. In terms of N application rate, the above ground plant fresh weight was significantly different at 8, 10, and 14 WAP. Vos and Biemond (1992) started that the growth of normal potato plants is extremely responsive to nitrogen fertilization. N enhances sympodial growth and delays senescence, both of individual leaf and of the entire plant. It is evident that the above ground plant fresh weight increased with time and from control and low N application rate to high N application rate 170 kg N/ha treatment.

Interaction effects between C: N ratio and N application rate on above ground plant dry weight (ton/ha) were significant at 14 WAP (Table7). The above ground plant dry weight was higher on C: N ratio 17 and 24 with the rate of 170 N kg ha⁻¹ than the non-amended control. The above ground plant dry weight increased with increasing N application rate in all treatments (Table 7). At 10 WAP the highest value of above ground plant dry weight was the same on C: N ratio 16 and 17, and it was lower on C: N ratio 24, while the C: N ratio 22 had intermediate value (Table 6). In terms of N application rate, there was no effect on the above ground plant dry weight at 8 WAP. However, at 10 and 14 WAP, the above ground plant dry weight values for plot receiving 170 kg N ha⁻¹ were significantly higher than the plot of lower N application rates and non-amended control. Rather at al. (1999) also found that the dry matter yield increases as N application rate increases.

Interaction effects between C: N ratio and N application rate on LAI ($m^2 m^{-2}$) were significant at 14 WAP (which is presented in table 7). The LAI was higher on C: N ratio 17 and 24 with the rate of 170 N kg ha⁻¹ than the non-amended control. The LAI increased with increasing N application rate in all treatments (Table 7). The LAI were not affected by C: N ratio at 8 and 14 WAP (Table 6). In terms of N application rate, the LAI was significantly different at 8, 10 and 14 WAP. The potato plants receiving 170 kg N ha⁻¹ were significantly higher in LAI than others with lower N application rates and non-amended control. Similar results of Njam et al. (2010) found that the above-ground dry matter accumulation and leaf area index are mostly influenced by N fertilization.

	Above ground fresh weight (tor	•		Above ground dry weight (ton	•		LAI (m ² m ⁻²)	
	8 WAP ¹	10 WAP	14 WAP	8 WAP	10 WAP	14 WAP	8 WAP	10 WAP	14 WAP
C: N ratio (F) ²									
24	8.3	19.7 a	27.1	0.74	1.88 a	2.50	0.49	1.09 a	1.80
22	9.0	22.1 ab	26.0	0.76	2.18 ab	2.40	0.52	1.29 ab	1.72
17	9.8	25.1 bc	26.6	0.87	2.46 bc	2.47	0.54	1.49 b	1.71
16	10.8	25.8 c	26.8	0.96	2.55 c	2.48	0.62	1.50 b	1.74
Significance ³	ns	**	ns	ns	**	ns	ns	*	ns
Application rate (R)								
0	7.4 a	12.8 a	14.7 a	0.63	1.35 a	1.40 a	0.40 a	0.65 a	0.96 a
57	8.9 a	19.6 b	20.2 a	0.78	2.02 b	1.85 a	0.52 ab	1.14 b	1.34 a
113	8.3 a	24.1 c	27.8 b	0.75	2.34 bc	2.54 b	0.49 a	1.37 bc	1.80 b
170	11.2 b	25.8 c	31.9 c	0.97	2.44 c	3.00 c	0.63 b	1.52 c	2.10 c
Significance	*	* * *	***	ns	**	***	*	**	***
F×R	ns	ns	ns	ns	ns	*	ns	ns	*

Table 6. Effect of C: N ratio (16, 17, 22 and 24) and N application rate (0, 57, 113 and 170 kg N ha⁻¹) on above ground plant fresh weight (ton ha⁻¹), above ground plant dry weight (ton ha⁻¹) and leaf area index (m² m⁻²) of potato.

¹ WAP = Week after planting.

² Different letters indicate significant differences according to Fisher's protected LSD-test (P < 0.05). ³ *, ** and *** refer to P values < 0.05, < 0.01 and < 0.001, respectively ; ns = not significant.

		Above ground plant dry weight (ton ha ⁻¹)	Leaf Area Index LAI $(m^2 m^{-2})$
		14 WAP	14 WAP
Treatment C: N ratio	Fertilizer rate (kg N ha⁻¹)		
Control	0	1.40 a	0.96 a
24	57	1.60 a	1.13 ab
24	113	2.72 e	1.98 d
24	170	3.18 fg	2.31 e
22	57	2.04 bc	1.41 bc
22	113	2.64 e	1.87 d
22	170	2.53 de	1.89 d
17	57	1.74 ab	1.33 bc
17	113	2.18 cd	1.46 c
17	170	3.48 g	2.35 e
16	57	2.02 bc	1.49 c
16	113	2.62 e	1.88 d
16	170	2.82 ef	1.85 d
LSDs		0.372	0.286

Table7. ANOVA results showing the interaction between C: N ratio (16, 17, 22 and 24) and N application rate (0, 57, 113 and 170 kg N ha⁻¹) on above ground plant dry weight and Leaf Area Index (LAI).

The interaction between C: N ratio and N application rate were significant. Different letters indicate significant differences according to Fisher's protected LSD- test (*P* < 0.05).

3.5 Fresh yield and agronomic nitrogen efficiency (ANE)

The fresh yield was affected by C: N ratio. The total tuber yield increased by decreasing the C: N ratio although there was no big difference (Figure 3). The total tuber yield was higher on low C: N ratio (16 and 17) whereas the highest C: N ratio (24 and 22) had the lowest tuber yield which was almost the same (Figure 3). This may be caused by early N availability from low C: N ratio to the potato plant during initial crop growth. In terms of N application rates, there was a linear relationship between N application rate and potato tuber yield (Figure 4). The treatment of 170 kg N ha⁻¹ showed the highest fresh yield (47.5 ton/ha) than the non-amended control (30.5 ton/ha) while the treatments with 57 and 113 kg N ha⁻¹ had intermediate with 35.8 and 40.5 ton/ha respectively. Maier et al. (1994) found an increase in tuber yield with increasing the rate of applied N.

In terms of marketable yield, use of low C: N ratio increased the marketable yield compared to high C: N ratio (Table 8). Regarding N application rate, the marketable yield was highest for 170 kg N ha⁻¹ and lowest for the control, with 57 and 113 kg N ha⁻¹ having intermediate values (Table 8). Gabr and Sarg (1998) also reported that nitrogen contributes to the increasing of the number of tubers by enhancing individual stem vigour. A similar result of Jamaati-e-Somarin et al. (2010) reported that with increasing nitrogen application, the number of stolons including tuber, the number of tubers and consequently yield increases. It is evident that in the current experiment the yield increased on the treatments with low C: N ratio and high N nitrogen application rate. This may be caused by early N availability in the high amount on the treatments with low C: N ratio and high N application rate during initial crop growth.

Regarding tuber grading, the C: N ratio did not affect the yield of small and cull tubers. However, there was a significant effect on the yield of large tubers (Table 8). In terms of N application rate, there was no significant effect on yield of cull tubers. The significant effect was observed for a yield of large tubers (> 40 mm) and small tubers (15- 40 mm). The non-amended control and plots received low N application yielded higher small tubers than the plots with higher N application. For the yield of large tubers, the 170 kg N ha⁻¹ treatment yielded higher compared to the other treatments while the non- amended control was the lowest. Ojala et al. (1990) stated that low N availability during the early growing season of potato, tuber initiation, and late tuber bulking can reduce total yield and yield of large tubers. Total tuber yield, marketable yield, and yield of large tubers were increased with increasing N application rates. These results are consistent with the previous reports (Jenkis and Nelson 1999, Maier et al. 1994 and Semiha 2009).

Agronomic nitrogen efficiency (ANE) was not affected by either the C: N ratio or N application rate (Table 8). The value of ANE tended to be highest for the lowest C: N ratio as well as for the highest N application rate. However, it was expected that the values of agronomical N efficiency become low at high N application rates, as Jamaati-e-Somarin et al. (2010) stated that the efficiency decreases at higher N input levels as factors other than N become more limiting to crop production.

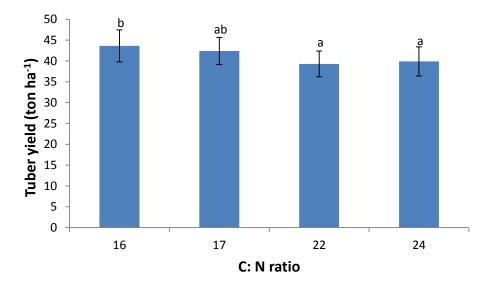


Fig. 3 Effect of C: N ratio on final potato tuber yield (ton ha⁻¹).

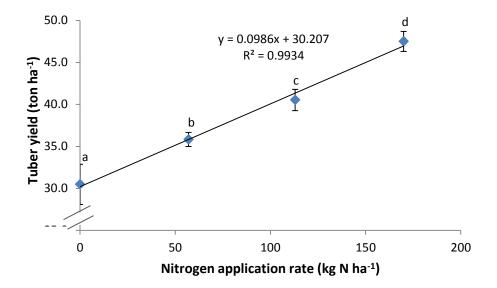


Fig. 4 Relationship between nitrogen application rate and final potato tuber yield.

		Tuber grading (ton/ha⁻¹	$)^{1}$		ANE (kg /kg)
	Marketable yield (> 15 mm)	Large (> 40 mm)	Small (15-40 mm)	Culls	
C: N ratio (F)					
24	37.7 ab	30.6 ab	7.1	2.2	80
22	37.3 a	29.8 a	7.5	2.0	74
17	40.7 bc	33.7 b	6.9	1.7	108
.6	41.3 c	33.8 b	7.5	2.3	115
Significance ²	*	*	ns	ns	ns
pplication rate (R) ³				
)	29.1 a	20.6 a	8.5 b	1.4	-
7	33.9 b	25.7 b	8.2 ab	1.9	94
.13	38.5 c	31.2 c	7.2 a	2.1	89
70	45.4 d	39.0 d	6.4 a	2.1	100
ignificance	***	***	*	ns	ns
-×R	ns	ns	ns	ns	ns

Table 8. Effect of C: N ratio (16, 17, 22 and 24) and N application rate (0, 57, 117 and 170 kg N ha⁻¹) on marketable yield (ton ha⁻¹), tuber grading (ton ha⁻¹) and agronomic nitrogen efficiency (ANE) of potato.

¹Tuber grading : Small = 15-40 mm ; Large = > 40 mm ; Culls = tubers with damages and or infestation regardless of their size. Marketable yield = Small and large tubers.

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

³ Different letters indicate significant differences according to Fisher's protected LSD-test (P < 0.05).

3.6 Tuber quality

The effect of C: N ratio on tuber quality (i.e., specific gravity and starch content) was not significant (Table 9). In terms of N application rate, there was a small effect of N application rate on tuber quality. The highest value of tuber specific gravity was observed on lower N application rate and on the non-amended control compared to high N application rate treatments. The specific gravity decreases with increasing nitrogen availability (Ojala et al. 1990). Kunkel and Holstad (1972) also reported that specific gravity of potato often decreased with increasing level of N.

Regarding the starch content, the lowest N application rate and the non-amended control yielded slightly higher starch content in tubers than the highest N application rate. These results are consistent with the one of Tein et al. (2014) also reported that the starch content was lower on large tuber compared to the small tuber. It is evident that increasing N application rate increases large tuber but decreases the specific gravity and starch content of potato. Similar results of Drakopoulos et al. (2014) also found the highest specific gravity and starch content on the non-amended control and low N application treatments compared to the treatments with high N application in organic potato production. Westermann et al (1994) also reported similar effects of N on starch content of potato.

	Tuber specific gravity (-)	Tuber starch content (%) ¹
C: N ratio (F)		
24	1.07	13.1
22	1.07	13.2
17	1.08	13.3
16	1.07	13.2
Significance ²	ns	ns
Application rate (R) ³		
0	1.08 b	13.6 b
57	1.08 b	13.6 b
113	1.07 a	13.2 a
170	1.07 a	12.9 a
Significance	**	**
F × R	ns	ns

Table 9. Effect of C: N ratio (16, 17, 22 and 24) and application rate (0, 57, 113 and 170) on tuber
quality, i. e. a specific gravity (-) and starch content (%) of potato tubers.

¹ According to Simmonds, 1977 : Starch content = $-1.39 + 0.196 [1000 \times (specific gravity-1)]$.

²Different letters indicate significant differences according to Fisher's protected LSD-test (P < 0.05).

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

3.7 Prediction of final tuber yield

For practical purposes, it might of interest to sort out if there exist simple indicators which correlate well with the final tuber yield. For this purpose, regression analyses were carried out between above ground crop growth measures, ANE, and final tuber yield. The results are presented in Table 10 and Appendix 3. According to R-squared, R-squared adjusted and *P* value, it is evident that emergence date, above ground fresh weight, above ground dry weight, leaf area index and ANE were not suitable to predict the final tuber yield (Table 10). However, leaf chlorophyll content was the best predictor of the final tuber yield since at 13 WAP the linear and quadratic regressions were 80% and 79% of the total tuber yield, respectively (Table 10). The quadratic relationship between leaf chlorophyll content at 13 WAP and final tuber yield is presented in Figure 5. Goudriaan and Monteith (1990) stated that LAI is closely correlated with a light interception and therefore with assimilating production and crop growth rate. Daugthry et al. (2000) reported that chlorophyll content of the leaf is closely correlated with the plant N status. Thus, lower SPAD-index value among the treatments is indicative of lower leaf N content which may influence the decrease in final tuber yield. Similar results, Semiha (2009) found that the total yield of potato significantly correlated with leaf chlorophyll content.

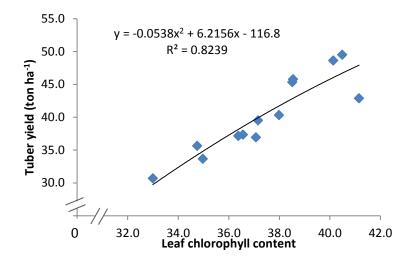


Fig. 5 Quadratic relationship between leaf chlorophyll content at 13 WAP and potato tuber yield (ton ha⁻¹).

Table 10.	Prediction	of final	tuber v	vield
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	Regression	R ²	R ² Adjusted	Equation	Significance
Emergence date			·		
	Linear	0.146	0.068	y = 30.59 + 0.1191x	ns
	Quadratic	0.290	0.147	$y = -7.6 + 1.053x - 0.00536x^2$	ns
Leaf chlorophyll content	t				
7 WAP ²	Linear	0.093	0.011	y = 129.8 – 1.76x	ns
	Quadratic	0.128	nd ³	$y = -3024 + 123x - 1.23x^2$	ns
9 WAP	Linear	0.095	0.013	y = -60.8 + 2.37x	ns
	Quadratic	0.226	0.071	$y = 5701 - 268x + 3.16x^2$	ns
11 WAP	Linear	0.673	0.643	y = - 53.6 + 2.08x	***
	Quadratic	0.674	0.609	$y = -155 + 6.5x - 0.049x^{2}$	**
13 WAP	Linear	0.821	0.804	y = -42.4 + 2.207x	***
	Quadratic	0.824	0.789	$y = -117 + 6.22x - 0.054x^2$	***
Above ground fresh wei	ight				
3 WAP	Linear	0.433	0.382	y = 22.36 + 1.921x	*
	Quadratic	0.489	0.387	$y = 57.5 - 6.08x + 0.437x^2$	**
10 WAP	Linear	0.691	0.663	y = 17.58 + 1.013x	***
	Quadratic	0.710	0.652	$y = 31.4 - 0.39x + 0.0335x^2$	**
14 WAP	Linear	0.693	0.665	y = 20.82 + 0.775x	***
	Quadratic	0.694	0.632	$y = 18.4 + 0.96x - 0.004x^2$	**
Above ground dry weigh	ht			· · · ·	
3 WAP	Linear	0.459	0.410	y = 21.54 + 22.9x	*
	Quadratic	0.490	0.388	$y = 52.1 - 57x + 49.6x^2$	*
10 WAP	Linear	0.526	0.483	y = 19.14 + 9.62x	**
	Quadratic	0.547	0.457	$y = 36.6 - 8.7 x + 4.55 x^2$	*
14 WAP	Linear	0.686	0.657	y = 21.68 + 7.8x	***
	Quadratic	0.687	0.625	$y = 18.3 + 10.8x - 0.63x^2$	**
Leaf Area Index				·	
8 WAP	Linear	0.434	0.383	y = 21.58 + 35x	*
	Quadratic	0.507	0.409	$y = 65.1 - 140x + 168x^2$	*

10 WAP	Linear	0.618	0.584	y = 22.16 + 14.01x	**
	Quadratic	0.619	0.543	$y = 20.6 + 16.6x - 1.01x^2$	**
14 WAP	Linear	0.636	0.603	y = 21.95 + 10.86x	**
	Quadratic	0.642	0.571	$y = 15.4 + 19.2x - 2.49x^2$	**
ANE	Linear	0.259	0.185	y = 30.59 + 0.1191x	ns
	Quadratic	0.496	0.384	$y = -7.6 + 1.053x - 0.00536x^2$	*

 1 *, ** and *** refer to *P*-values < 0.05, < 0.01 and < 0.001, respectively ; ns = not significant. ² WAP = weeks after planting.

3 nd = not determined.

4. Conclusions

The aim of the current research was to investigate the effect of C: N ratio (16, 17, 22, and 24) and nitrogen application rate (57, 113 and 170 kg N ha⁻¹) on above ground crop performance, tuber yield and agronomic nitrogen efficiency in organic potato production. There were significant differences between low and high C: N ratio and between low and high N application rate on above ground plant performance and tuber yield of potato. Regarding C: N ratio, the treatment with high C: N ratio recorded lower value of above ground plant growth and tuber yield compared to the treatment with low C: N ratio. This confirmed partly the hypothesis stated that the use of silage with low C: N ratio (16) will result on better above ground crop growth, tuber yield, and agronomic nitrogen efficiency because of its earlier release of N. It is evident that the C: N ratio didn't affect significantly ANE, plant emergence, tuber grading (small and cull tubers) and tuber quality (specific gravity and starch content). From 9 to 13 WAP, the leaf chlorophyll content was lower on the treatments with high C: N ratio compared to the treatments with low C: N ratio probably due to immobilization. Therefore, this may provide insight into the best quality of grass-clover silage (C: N ratio) required for optimal N release to synchronise with crop demand. In terms of N application rate, the hypothesis was also partly confirmed. The high nitrogen input increased leaf chlorophyll content, above ground plant fresh weight, above ground plant dry weight, LAI, fresh yield, and large tuber yield. However, N application rate didn't affect the plant emergence, tubers graded as culls and ANE. The values of ANE increased with increasing N application rate although they were not statistically different. Regarding the fresh yield, the 170 kg N ha⁻¹ treatment differed significantly from the lower N application rates and the non-amended control. On the other hand, the use of low N application rate increased the fraction of small tubers (15-40 mm) and had a positive effect on tuber quality (specific gravity and starch content). Thus, the hypothesis was partly confirmed as C: N ratio didn't show any effect on tuber quality. At 13 WAP, the results showed that leaf chlorophyll content was the best predictor for the final yield as it accounted for almost 80% of the overall yield variability. This was not expected as it was hypothesized that LAI would be the best total yield predictor compared to the leaf chlorophyll content. The use of grass-clover material with low C: N ratio and high N application rate may result in faster N release which influences crop performance and tuber yield. Therefore, it may be stated that high N application rate with lower C: N ratio increases the above-ground crop performance and tuber yield in organic potato production. However, high N application rate slightly decreased the tuber quality.

5. Recommendations

Based on the results of this study, it appears that the treatment with low C: N ratio and high N application rate performed better regarding the above ground plant growth and tuber yield. It is better to take into account for the grass clover silage quality (C: N ratio) used as a nutrient source to be able for supplying to the plant the essential nutrients needed in term of amount and time in order to synchronize N release to potato crop demand. Since there is grass-clover silage with high C: N ratio (24), it may be better to apply them few weeks before planting as immobilization occurred due to the high C: N ratio. The N content of the silage material is required for providing the nutrient needed by the crop, thus pasture management of grass-clover can be improved to ensure the best quality of the silage which will be used for supplying the N needed in organic potato production. Finally, as potato performed better in terms of above ground crop growth measurements and tuber yield with a high amount of nitrogen application rate, further researches are needed to evaluate the level of N leaching.

References

- ASKEGAARD, M., ERIKSEN, J. 2007. Growth of legume and nonlegume catch crop and residual N effects in spring barley on coarse sand. J. Plant Nutr. Soil Sci. 170, 773-780.
- BALL, B. C., WATSON, C. A., CRICHTON, I, 2007. Nitrous oxide emission from cereal growth, N recovery and soil nitrogen status after ploughing organically managed grass/clover swards. Soil use manages. 23, 145-155.
- BISHNU, H. A., AND KRISMA, B., K. 2006. Effect of potassium on potato tubers production in acid soils of Malepatan, Pokhara. Nepal Agricultural Research Journal, pp.7
- BOWEN, W. T. 2003. Water productivity and potato cultivation. In water productivity in agriculture: Limits and opportunities for improvement Ed. J. W. Kinje, R. Baker and D. Molden. CAB international, pp. 229-238.
- CANALI, S., CIACCIA, C., TITTARELLI, F. 2012. Soil fertility management in organic potato: The role of green manure and amendment applications, in: He, Z., Larkin, R., Honeycutt, W. (Eds.), Sustainable potato production: Global case studies. Springer Netherlands, pp. 453-469.
- CBS, PBL, WAGENINGEN UR, 2015. Biologische landbouw: aantal bedrijven en areaal, 1998-2014 (indicator 001, versie 14, 21 mei 2015).
- CHERR, C. M., SCHOLBERG, J. M. S., AND MCSORLEY, R. 2006. Green manure approaches to crop production: *A Synthesis Agronomy Journal*, 98, 302-319.
- DAUGHTRY, C., WALTHALL, C., KIM, M., DE COLSTOUN EB AND MCMURTREY, J. 2000. Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. Remote Sensing of Environment, 74, 229-239.
- DRAKOPOULOS, D., SCHOLBERG, J. M. S., LANTINGA, E. A., AND TITONNEL, P. A. 2014. Influence of reduced tillage and fertilization regime on crop performance and nutrient utilization regime of organic potato in The Netherlands. Master thesis in Farming System Ecology, Wageningen University, Wageningen, The Netherlands.
- ELFSTRAND, S., BATH, B., AND MARTENSSON, A. 2007. Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. *Applied soil ecology* 36, 70-82.
- Eurostat, 2014. Area under organic farming. Eurostat, Luxemburg.
- GABR, S. M & SARG, S. M. 1998. Response of some new potato cultivars grown in sand soil to different nitrogen levels. Alexandria journals of Agricultural Research 43: 33-41.
- GOUDRIAAN, J., MONTEITH, J. L. 1990. A Mathematical Function for Crop Growth Based on Light Interception and Leaf Area Expansion. Ann. Bot. 66, 695-701.
- GÜLER, S., AND GÜZEL N. 1999. Effect of varying level of nitrogen and potassium concentration in the nutrient solution on the yield and leaf composition of drip-fertilized tomatoes. Acta Hort. 506, 81-85.

- HA, K. V., MARSCHNER, P., BÜNEMANN, E.K. 2008. Dynamics of C, N, P and microbial community composition in particulate soil organic matter during residue decomposition. Plant Soil 303, 253-264.
- JAMAATI-E-SOMARIN, S., ZABIHI-E-MAHMOODABAD, R., YARI, A. 2010. Response of Agronomical, Physiological, Apparent Recovery, Nitrogen Use Efficiency and Yield of potato tuber (*Solanum tuberosum* L.), to Nitrogen and Plant Density. Amer- Eurasian J Agri Env. Sci 9, 16-21.
- JARREL, W. M., AND BEVERLY, R. B. 1981. The dilution effect in plant nutrition studies. Advanced Agron. 34, 197-222.
- JENKIS, P. D., AND NELSON, D. G. 1992. Aspects of nitrogen fertilizer rate on tuber dry matter content of potato cv. Record. Potato Res. 35, 127-132.
- KARAM, F., ROUPHACI, Y., LAHOUD, R., BREIDI, J., COLL, G. 2009. Influence of Genotypes and potassium application rates on yield and potassium Use Efficiency of potato. J Agro, 8, 27-32.
- KUEPPER, G., THOMAS, R., AND EARLES, R. 2001. Use of baking soda as a fungicide. National centre for appropriate technology, Fayetteville, Arizona, USA. Available at: <u>http://attra.ncat.org/attra-pub/bakingsoda.html</u>.
- KUNKEL, R., HOLSTAD, N. 1972. Potato chip color, specific gravity and fertilization of potatoes with N-P-K. Am J Potato Res 49, 43-62.
- LANTINGA, E. A., GÁBORČÍK, N., DIRKS, B. O. M., 1996. Ecophysiological aspects of herbage production in grazed and cut grassland. Grass land and land use system 16th EGF, 151-161.
- LAWLOR, D. W., 2001. Carbon and nitrogen assimilation in relation to yield: Mechanisms are the key to understanding production systems. Journal of Experimental Botany, 53, 773-787.
- LAZA, R. C., BERGMAN, B., VERGARA, B. S. 1993. Cultivar differences in growth and chloroplast ultrastructure in rice as affected by nitrogen. Journal Experimental Botany 44, 1643-1648.
- LESZCZYŃSKI, W., LISISŃKA, G. 1988. Influence of nitrogen fertilization on the chemical composition of potato tubers. Food chem. 28, 45-52.
- MAIER, N. A., DAHLENBURG, A. P., AND WILLIAMS, C. M. J. 1994. Effects of nitrogen, phosphorus, and potassium on yield, specific gravity, crisp color and tuber chemical composition of potato (*Solanum tuberosum L.*) cv. Kennebec. Aust. J. Exptal. Agric. 34, 813-824.
- MARKWELL, J., OSTERMAN, J. C., AND MITCHELL, J. L. 1995. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. Photosynthesis Research, 46, 467-472.
- MÜLLER, T., VON FRAGSTEIN AND NIEMSDORFF, P. 2006. Organic fertilizers derived from plant material Part I: Turnover in soil at low and moderate temperatures. J. Plant Nutr. Soil Sci. 169, 255-264.
- NAJM, A. A., FAZELI, F., TAGHI DARZI, M., SHAMORADY, R. 2010. Effect of utilization of organic and inorganic nitrogen source on the potato shoots dry matter, leaf area index and plant height, during the middle stage of growth. World Acad. Sci. Eng. Technol. 47, 900-903.

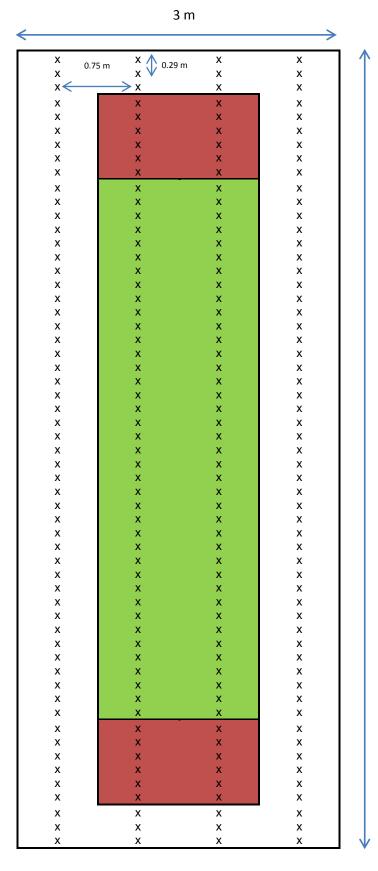
NIEMSDORFF PVFU AND MULLER, T. 2006. Plant based organic fertilizer. A viable nutrient source for organic market gardens. *Acta Horticulturae*, 255-260.

- OLESEN, J. E., ASKEGAARD, M., RAMSMUSSEN, I. A. 2009. Winter cereal yields as affected by animal manure and green manure in organic arable farming. *European Journal of Agronomy* 30, 119-128.
- OJALA, J. C., STARK, J.C., KLEINKOPF, G.E. 1990. Influence of irrigation and nitrogen management on potato yield and quality. Am. Potato J. 67, 29-43.
- RASMUSSEN, J., SØEGAARD, K., PIRHOFER-WALZL, K., ERIKSEN, J. 2012. N₂ fixation and residual N effect of four legume species and four companion grass species, Eur. J. Agron 36, 66-74.
- RATHER K., SCHENK, M., EVERAARTS, A., VETHMAN, S. 1999. Response of yield and yield of cauliflower varieties (*Brassica oleracea var. botrytis*) to nitrogen supply. *Journal of Horticultural Science and Biotechnology*, 74, 658-664.
- SANCHEZ, P. A., SHEPHERD, K. J., SOULE, M. J., PLACE, F. M., BURESH, R. J., IZAC, A. M. I., MOKWUNYE, A. U., KWESIGA, F. R., NDIRITU, C. G., AND WOOMER, P. L. 1997. Soil fertility replenishment in Africa: An investment in natural resource capital. In: Replenishing soil fertility in Africa. pp. 1-46.
- SCHOLBERG, J. M. S., TER BERG, C., STAPS, J. J. M., VAN STRIEN, J. 2009. Minder en anders bemesten: voordelen van maaimeststoffen voor de teelt van najaarsspinazie: resultaten veldproef bij Joost van Strien in Ens. Louis Bolk Intituut (Rapport/ Louis Bolk Instituut nr. 2010-007 LbP, 51.
- SEMIHA, G. 2009. Effects of nitrogen on yield and chlorophyll of potato (*Solanum tuberosum L.*) cultivars. Bangladesh J. Bot. 38, 163-169.
- SIMMONDS, N. W. 1997. Relation between specific gravity, dry matter content and starch content of potatoes. Potato Research, 20, 137-140.
- STEPHEN, D., JACKSON, A. 1999. Multiple signalling pathways control tuber induction in potato plant physiology, 119, 1-8.
- SORENSEN, J. N., AND THORUP-KRISTENSEN, K. 2011. Plant-based fertilizers for organic vegetable production. J Plant Nutr Soil Sc 174, 321-332.
- TABATABAI, A., ARCHAD, M., NADERI, M. R. 2014. Effect of bio-fertilization on the yield of potato cultivar marfona. International journal of Advanced Biological and Biomedical research 2, 272-278.
- TEIN, B., KAUER, K., EREMEEN V., LUIK, A., SELGE, A., AND LOIT, E. 2014. Farming systems affect potato (*Solanum tuberosum L.*) tuber and soil quality. Field crop Research, 156, 1-11.
- TERRA, B. 2014. How does cut-and-curry green manure fertilization affect above-ground crop growth, yield and agronomic nitrogen efficiency in organic potato production? BSc Thesis in Farming System Ecology in Wageningen University, Wageningen, The Netherlands.

- THORUP-KRISTENSEN, K. 2006. Root growth and nitrogen uptake of carrot, early cabbage, onion and lettuce following a range of green manures. Soil use and management 22, 29-38.
- VAN HEEMST, H. D. J. 1986. The distribution of dry matter during growth of potato crop. *Potato Research* 29: 55-66.
- VOS, J. & BIEMOND, 1992. Effects of nitrogen on the development and growth of the potato plant. Leaf appearance, expansion growth, life spans of leaves and stem branching: *Annals of Botany* 70: 27-35.
- WESTERMANN, D. T., JAMES, D. W., TINDALL, T. A., HURST, R. L. 1994. Nitrogen and Potassium fertilization of potatoes sugars and starch. Amer Potato J 71, 433-451.
- ZEBATH, B. J., YOUNIE M, PAUL, J. M., BITTMAN, S., 2002. Evaluation of leaf chlorophyll index making fertilizer nitrogen recommendations for silage corn in a fertility environment. Comm. Soil Sci. Plant Anal. 33, 665-684.

Appendixes

Appendix 1 Plot layout



15 m

Appendix 2

	Total yield	
C: N ratio (F)		
24	39.9 a	
22	39.3 a	
17	42.4 ab	
16	43.6 b	
Significance ¹	*	
Application rate $(R)^2$		
0	30.5 a	
57	35.8 b	
113	40.5 c	
170	47.5 d	
Significance	***	
F×R	ns	

Table showing the effect of C: N ratio (16, 17, 22 and 24) and N application rate (0, 57, 117 and 170 kg N ha⁻¹) on fresh yield (ton ha⁻¹).

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

² Different letters indicate significant differences according to Fisher's protected LSD-test (P < 0.05).

Appendix 3. Prediction of final tuber yield

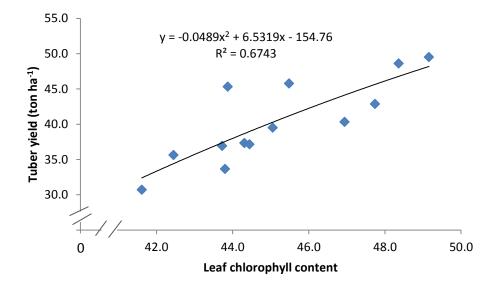


Figure showing the quadratic relationship between leaf chlorophyll content at 11 WAP and potato tuber yield (ton ha⁻¹).

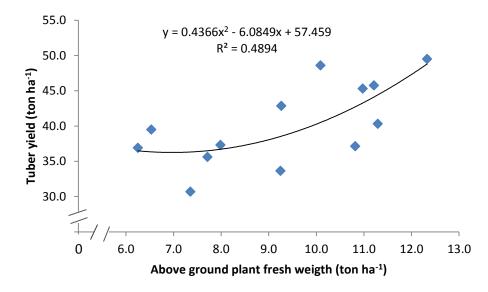


Figure showing the quadratic relationship between above ground plant fresh weight at 8 WAP and potato tuber yield (ton ha⁻¹).

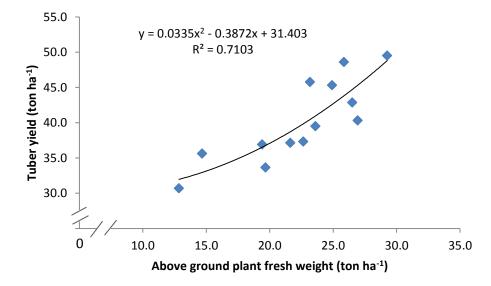


Figure showing the quadratic relationship between above ground plant fresh weight at 10 WAP and potato tuber yield (ton ha⁻¹).

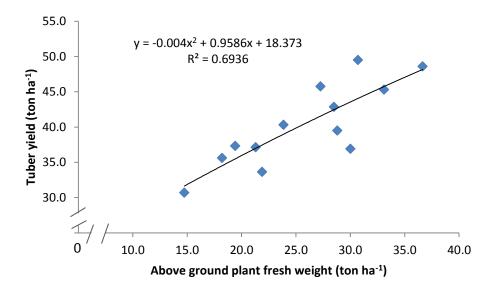


Figure showing the quadratic relationship between above ground plant fresh weight at 14 WAP and potato tuber yield (ton ha⁻¹).

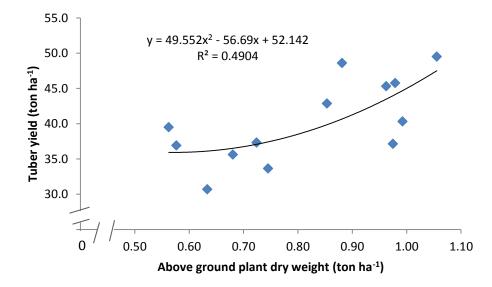


Figure showing the quadratic relationship between above ground plant dry weight at 14 WAP and potato tuber yield (ton ha⁻¹).

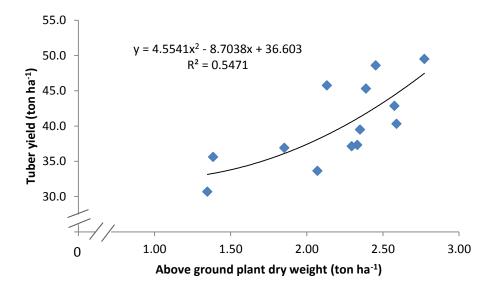


Figure showing the quadratic relationship between above ground plant dry weight at 10 WAP and potato tuber yield (ton ha⁻¹).

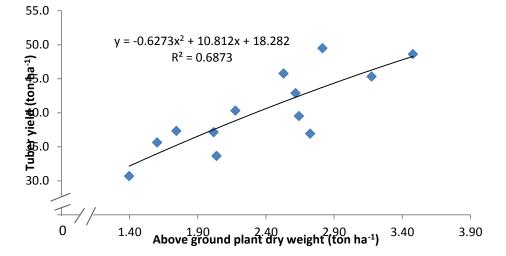


Figure showing the quadratic relationship between above ground plant dry weight at 14 WAP and potato tuber yield (ton ha⁻¹).

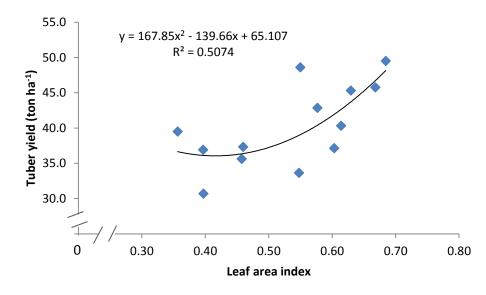


Figure showing the quadratic relationship between leaf area index at 8 WAP and potato tuber yield (ton ha⁻¹).

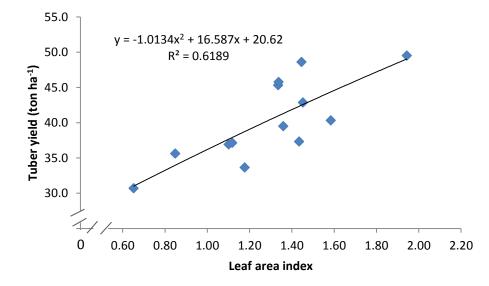


Figure showing the quadratic relationship between leaf area index at 10 WAP and potato tuber yield (ton ha⁻¹).

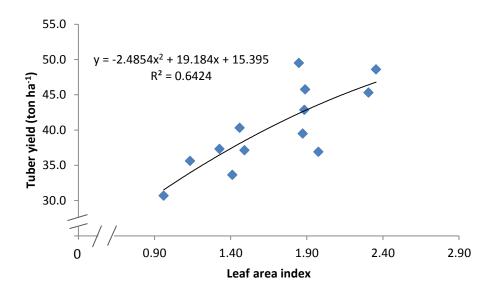


Figure showing the quadratic relationship between leaf area index at 14 WAP and potato tuber yield (ton ha⁻¹).

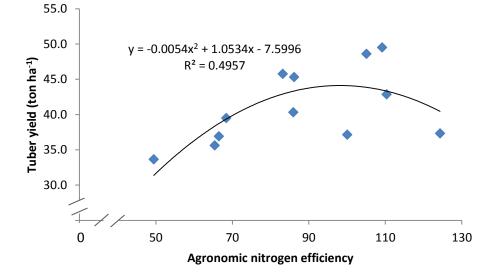


Figure showing the quadratic relationship between agronomic nitrogen efficiency and potato tuber yield (ton ha⁻¹).