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Optimization of nitrogen nutrition of cauliflower intercropped with clover and in rotation with lettuce

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ABSTRACT

Cauliflower (*Brassica oleracea* var. *botrytis* L.) requires large amounts of nitrogen (N) fertilizer to produce high yields. The intercropping of cauliflower with a leguminous species may help farmers to reduce N fertilizer rates, production costs and environmental pollution. Moreover, the recycling of crop residues can contribute to the nutritional support of subsequent crops in a rotation. In this study, cauliflower was cultivated in year one either alone or intercropped with annual clover (*Trifolium resupinatum* L.), using four N fertilization rates: 0, 75, 150, and 300 kg N ha^{-1} (referred to as N0, N1, N2, and N3 respectively). Following crop residue incorporation in year two, iceberg lettuce (*Lactuca sativa* var. *capitata* (L.) Janchen) was cropped on the same field without the supply of N fertilizer to assess the effect of cauliflower and clover residues as well as of residual N fertilizer rates on the growth and N uptake of lettuce.

The presence of clover did not affect the marketable yields of the intercropped cauliflower, which were similar to the cauliflower sole-cropped. The N1 and N2 fertilizer rates maximized both growth and N uptake of the intercropped cauliflower, while with the N3, crop development was limited. In the N0 plot, the legume competed for N and depressed growth and N absorption of cauliflower. The sole-cropped cauliflower was, on the contrary, unaffected by the N fertilizer rates. The iceberg lettuce cultivated in succession benefited from the presence of clover in plots N1 and N2. When succeeded to the sole-cropped cauliflower, however, the iceberg lettuce produced less biomass and absorbed less N. Results from this study suggest that the intercropping system cauliflower-clover can be a sustainable tool to optimize N input and reduce N fertilizer requirements for the successive crop.

1. Introduction

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is one of the most commonly grown brassica species worldwide, with more than 15,000 ha cultivated in Italy alone (ISTAT, 2017). To obtain high yield and curd quality, this crop consistently requires a generous supply of nitrogen (N) to achieve a soil availability of 250–300 kg N ha^{-1} (Everaarts, 1993; Everaarts et al., 1996), although uptake rates are often in the range of 130–150 kg N ha^{-1} yr^{-1} (Di Gioia and Santamaria, 2009). Cauliflower and many other vegetable crops can be cultivated alone as a sole-crop or intercropped with other species. Most N taken up by cauliflower is absorbed during the 50 days prior to harvest (Welch et al., 1987; Thompson et al., 2000).

In the intercropping systems, the degree of complementarity and competitiveness of the involved species are pivotal to ensure the ecological and economical sustainability of the system (Tofinga et al., 1993; Lithourgidis et al., 2011). Andersen et al. (2005) studying the interactions among pea, barley, and rape, found out that barley was more competitive for N uptake against pea and rape due to its faster growth during the first development period. Yildirim and Guvenc (2005) showed an increase in the productivity of cauliflower when intercropped with onion, lettuce, or bean, but not with radish, due to allelopathic effects of their root exudates.

Little information is available regarding the intercropping of cauliflower with legumes. A legume species intercropped with cauliflower can reduce N losses compared to the sole-cropping system (Kristensen et al., 2014). Recently, Xie and Kristensen (2016) demonstrated that the combination of a legume intercrop (clover) and low N fertilization could limit nitrate leaching and maintain optimal cauliflower yields, possibly as a consequence of symbiosis with the N-fixing *Rhizobium*

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leguminosarum and because N is slowly released during the crop cycle (Borreani et al., 2003; Crews and Peoples, 2004). In intercropped systems, legumes enhance the yield of the main crop and are a potential source of plant nutrients (Neamatollahi et al., 2013), but might compete for water, light and nutrients (Båth et al., 2008). Therefore, when planning an optimal intercropping system, it is fundamental to evaluate the species physiology and growth requirements. The presence of a legume in intercropping systems can be beneficial either to the main crop and/or the crops cultivated in rotation. Besides having an effect on N availability for the crop following in succession due to harvest residues (Unkovich, 2008), intercropped legumes can improve the soil conditions, prevent the development of weeds, and improve soil aggregate stability (McVay et al., 1989).

Cauliflower may leave a considerable amount of soil residual N at harvest in the crop residues (from 90 to 221 N kg ha^{-1}, van den Boogaard and Thorup-Kristensen, 1997; Everaarts, 2000; Chaves et al., 2007). The contemporary presence of N fertilizer and crop residues, the environmental conditions, and the timing of biomass incorporation into the soil influence the residue decay rate and the amount of N released and made available to other crops cultivated in succession (Greenwood et al., 1996; Quemada and Cabrera, 1997; Mitchell et al., 2000).

Against this background, there is a need to increase our knowledge on how to make the N management in cauliflower production more environmentally friendly. In this experiment, we contrasted different N rates to cauliflower crops with the presence/absence of a legume as intercropped species, to assess the N uptake rates (1), to produce evidence of the benefit of the presence of the legume on the intercropped cauliflower (2), as well as on the N nutrition of lettuce plants grown in succession (3). The lettuce crop is known as a suitable indicator of the residual effect of N fertilizers (Gianquinto and Borin, 1995). The study has been carried out in a mountainous environment (South Tyrol, northern Italy) where the leguminous annual clover is planted to limit soil erosion and N leaching in winter, as well as a weed suppressant.

2. Material and methods

2.1. Experiment 1: growth and N uptake of cauliflower as affected by intercropping and N supply

Experiment 1, carried out in summer-autumn 2015, aimed at testing the effect of clover as an intercrop with cauliflower at increasing N supply rates. The experiment was carried out at the experimental farm of the Laimburg Research Center in Oris/Eyrs (Bolzano/Bozen, Val Venosta, South Tyrol - Italy), 46°37′23.2″ N, 10°38′53.4″ E, 900 m a.s.l., on a sandy-silt soil. Soil analyses were carried out before transplanting as in Zanotelli et al. (2014) (Table 1). The meteorological data were recorded on location; on average, rainfall was concentrated between May and October 2015 (60–80 mm per month) and between April and August 2016 (60–100 mm per month). Air temperature ranged between 17–20 °C during the summer and reached -3 - + 2 °C in the following winter (Fig. 1).

The factorial experiment had a split-plot design with three blocks. The main plot factor was the N rate with four levels (N0, N1, N2, N3 = 0, 75, 150, 300 kg N ha^{-1} respectively), which was divided into subplots to accommodate the two cropping systems (sole-crop cauliflower and cauliflower intercropped with annual clover). Each experimental unit covered 9.5 m^2 and was separated by border rows.

On 16 April 2015, 12 t ha^{-1} of cow manure (~70 kg N ha^{-1}) was applied and ploughed into the soil. White cauliflower (*Brassica oleracea* var. *botrytis* L. cv. Seoul F1, VILMORIN) was sown in trays filled with a mixture of peat and vermiculite and placed in the greenhouse on 25 May 2015 for five weeks, when 4–5 true leaves were formed. Before transplanting, the soil was mechanically tilled and levelled and the experimental units were established. Plants were then transplanted at a distance of 0.65 m between rows and 0.42 m within row for a total of 36 plants per experimental unit, or 3.6 plants m^{-2}. Annual clover (*Trifolium resupinatum* L. cv. Gorby) was manually sown at a rate of 2.5 g of seeds per m^2 the day prior to cauliflower transplanting in order to obtain a uniform cover.

The N1, N2, and N3 treatments were side dressed with different fertilizer rates (ammonium nitrate, 27% N) equally split at two growth stages: 20 days after transplanting (vegetative stage) and 60 days after transplanting (reproductive stage). Manual weeding was carried out twice in the subplots without clover and once in the subplots with clover. The crop cycle was 80 days from transplanting to harvest.

Cauliflower curds were harvested at commercial maturity on 15 September 2015. The marketable yield was quantified by measuring the fresh weight of the curds comprising the white inflorescence and the lower part of the leaves surrounding the inflorescence (Everaarts, 2000). A sample of five plants of cauliflower was collected from the center of each experimental unit. The fresh weight of leaves, roots, stem, and curd was measured. Where present, clover was sampled from an area of 0.5 m^2: the aboveground biomass was harvested and roots collected from the 0–0.40 m soil layer after sieving. A subsample of each plant organ of both cauliflower and clover was weighed (on average: 35 g roots, 60 g leaves, 80 g stem,150 g curd), dried at 60 °C to a constant weight and weighed again (dry weight = DW). All collected subsamples were milled and analyzed for total N and carbon (C) concentration with an elemental analyzer (Flash EA 2000, ThermoFisher Scientific). After sampling, the remaining plant biomass was placed in the original experimental unit.

Crop residues (roots, stems, and leaves) were assessed in terms of DW, C, N content and data referred on a per-hectare basis. The C:N ratio of both residue types (cauliflower alone and cauliflower plus clover) was calculated.

On 15 October 2015, four soil cores were collected with a 30-mm-

Table 1
Chemical and physical properties of the soil.

Parameter	Value	Method
Soil type	Sandy silt	*VDLUFA Methodenbuch I D 2.1*
Organic carbon expressed as humus	2.80%	*Standard ISO*10694*: 1995 conversion factor 1.72*
pH (in $CaCl_2$)	7.7	*10:25 soil:water extraction followed by potentiometric determination of pH*
N Tot	0.19%	*Dumas combustion method. CHN elemental analyzer*
C Tot	1.86%	*Dumas combustion method. CHN elemental analyzer*
Phosphorous in CAL solution (P_2O_5)	2.6 mg/kg	*ÖNORM L 1087:2012 A5*
Potassium in CAL solution (K_2O)	1.8 mg/kg	*ÖNORM L 1087:2012 A5*
Magnesium in CAT solution (Mg)	5.3 mg/kg	*VDLUFA Methodenbuch I A 6.4.1*
Boron in CAT solution (B)	1.56 mg/kg	*VDLUFA Methodenbuch I A 6.4.1*
Manganese in CAT solution	49 mg/kg	*VDLUFA Methodenbuch I A 6.4.1*
Copper in CAT solution	9 mg/kg	*VDLUFA Methodenbuch I A 6.4.1*
Zinc in CAT solution	3 mg/kg	*VDLUFA Methodenbuch I A 6.4.1*

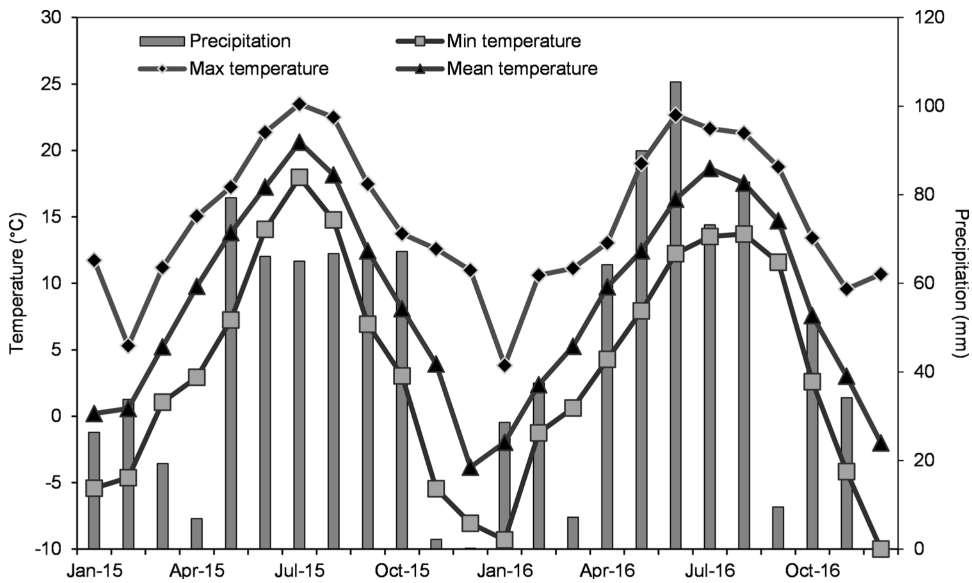

Fig. 1. Air temperature and precipitation data for Oris/Eyrs - January 2015 to December 2016.

diameter auger (Eijkelkamp, NL) from the upper 0–0.40 m soil layer of each experimental unit and their nitrate and ammonium concentration determined to assess the effect of treatments on residual soil mineral N. The collected soil samples were homogenized, a fresh subsample of 10 g was then transferred into a 500 ml flask, and 100 ml of KCl 2 M was added. Flasks were shaken on a horizontal shaker for 1 h. The supernatant was collected, filtered, transferred into a 50 ml tube, and centrifuged for 10 min at 3000 rpm. The analysis of ammonium and nitrate concentration was performed by a continuous flow AutoAnalyzer (AA-3; Bran + Luebbe, Norderstedt, Germany). In order to assess the dry weight of the soil, an additional fresh subsample of 10 g was collected from each sample and was oven dried at 65 °C until the weight remained constant.

2.2. Experiment 2: growth and N uptake of lettuce as affected by the preceding cropping systems

The residual effects of both N fertilizers and crop residue of cauliflower and annual clover have been assessed on the growth and N uptake of a lettuce crop (*Lactuca sativa* var. *capitata* (L.) Janchen) cultivated in succession on the same field. On 20 October 2015, aboveground crop residues of both clover and cauliflower, with known biomass and N content, were mechanically ground and left on the top of the soil until 17 May 2016, when the upper 0-0.30 m soil layer was mechanically tilled to incorporate the crop residues. On 4 May 2016, soil nitrate and ammonium concentration was analyzed as previously described. Experiment 2 had the same experimental design and treatments as Experiment 1. In order to evaluate the effect of the 2015 trial treatments on the biomass and N uptake of the 2016 crop, no fertilizers were applied either as a base dressing or during the crop cycle. On 15 April 2016, iceberg lettuce (cv. Umbrinas, Rikj Zwaan Zaadteelt en Zaadhandel B.V.) was sown in trays filled with a mixture of peat and vermiculite and placed in greenhouse until 20 May 2016. Seedlings were then transplanted with a planting density of 0.32 m between rows and 0.35 m within row, for a total of 8 plants m^{-2}. Manual weeding was carried out twice during the lettuce crop cycle, which lasted 55 days from transplanting to harvest. On 13 July 2016, at commercial firmness, the lettuces were harvested by cutting the head at stem level and removing the outermost leaves. A sample of three plants was collected from the center of each experimental unit, the fresh weight of leaves and roots was recorded and the marketable yield quantified. Subsamples of lettuce leaves and roots were collected, oven dried at 60 °C up to constant dry weight, milled and analyzed for total N concentration.

2.3. Data analysis

Data were subject to a split-plot ANOVA (Gomez and Gomez, 1984; de Mendiburu, 2017) including blocks, N treatment, and cropping systems treatment as model terms. Means were separated using Tukey HSD test (Hothorn et al., 2008) with $p \leq 0.05$. Before analysis, all data were checked for normality and homogeneity of the variance. Statistical analysis was carried out using R statistical software version 3.3.2 (R Core Team, 2016). Averages and standard errors were calculated. In accordance with the split-plot design statistical analysis, the interaction between the main plot and sub plot factors was first observed. As a second step, and whenever the interaction was not significant, the effects of the main plot factor and of the sub-plot factor were separately analyzed.

3. Results

3.1. Experiment 1: effects on growth and yield

Nitrogen rates significantly affected the marketable curd yields, while no difference between the two cropping systems and no interaction between cropping systems and N rates were found (Fig. 2). Yields increased from N0 to N1, did not change between N1 and N2 and then tended to decrease from N2 to N3.

N rates and cropping systems differently affected the biomass growth of above and below ground cauliflower organs (Table 2). Root and stem DW were lower in intercropped plots than in sole-cropped cauliflower, but they were unaffected by N rates. Curd DW progressively increased from N0 to N2, decreased from N2 to N3 regardless the cropping system, and it was always higher in sole-cropped plots than in intercropped plots. Regardless of the treatment, most plant DW was represented by leaf DW. Leaf DW and total plant DW were similarly affected by the treatments and the analysis of both datasets showed a significant interaction between N rates and cropping systems. When cauliflower was cropped alone, there was no effect of the N rates, whilst when intercropped, leaf and total biomass from N0 to N1 increased considerably and then they gradually decreased from N1 to N3. Cauliflower, both cropped alone and intercropped, produced similar total biomass when fertilized with N1 and N2. In unfertilized plots (N0) and

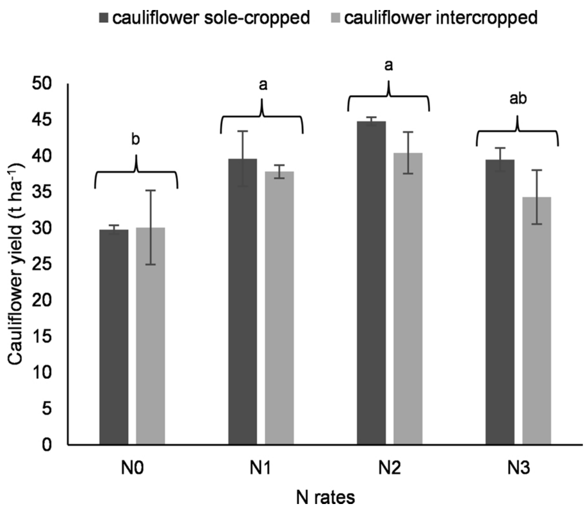

Fig. 2. Cauliflower yield as affected by N rates. Bars are averages; vertical lines represent the standard errors. Small letters indicate differences among N rates for $p \leq 0.05$.

in plots receiving the highest N rate (N3), a higher biomass was recorded when cauliflower was cropped alone than when it was intercropped.

The total biomass produced by clover averaged 280 g (DW) m^{-2} (S.E. ± 52.21) and was unaffected by the N rate (Table 1, Supplementary material).

3.2. Effects on nitrogen uptake

The N content of cauliflower roots and stem was unaffected by the N rate, but it was higher when cauliflower was cropped alone (Table 3). The amount of N contained in the curds gradually increased from N0 to N2, but decreased from N2 to N3 (Table 3). A significant interaction between N rate and cropping systems occurred for both leaf N and total N content. When cauliflower was cropped alone, the N rate did not affect neither the leaf N nor the total N content, but when it was intercropped, they both increased from N0 to N1. Increasing the N rate from N1 to N3 did not cause any further increase of total N content. Where no N was supplied (N0), the N content of cauliflower was higher if it was cropped alone rather than intercropped (Table 3).

The N content of clover averaged 5.2 g N m^{-2} (S.E. ± 1.05) and it was unaffected by the N rate (Table 1, Supplementary material).

3.3. Experiment 2: residual N fertility

Leaf N concentration was unaffected by the cropping system, but progressively increased with increasing N rates with statistically significant differences between N0 and N3 (Table 4). Both N rates and cropping systems significantly affected mineral N concentration of the soil sampled in October 2015 (Table 4). A significant interaction was also observed. The significant differences were mainly due to the N-NO_3^- concentration (data not shown), while N-NH_4^+ accounted only for some 5–20% of the total soil mineral N. In both cropping systems, the N content of the soil was higher in N3 as compared to the other N rates, which were not statistically different. In May 2016, the soil mineral N concentration was not statistically affected by treatments (Table 4). In N3 plots, the soil mineral N concentration was on average lower in May 2016 than in October 2015.

The total biomass of plant residues and their N content are reported in Table 5. The N rates had no effect on the biomass or on the N content of residues when cauliflower was sole-cropped. On the contrary, the cumulative biomass of cauliflower and clover in the intercropped plots and their N content increased from N0 to N1 and tended to decrease from N2 to N3. The N content of the cumulative residue amount of cauliflower and clover in the intercropped plots was higher than that of the sole-cropped cauliflower at N1 and N2 rates. In the intercropped plots, the fraction of N derived from clover residues on total N of residues ranged from 33% in N0 to 10% in N2.

The C:N ratios of cauliflower (both cropping systems) and clover residues are shown in Table 6. The C:N ratio of the cauliflower stem did not change among treatments, but differences were apparent from the root C:N ratio, which was lower in N3 than in N0 and N1. The interaction between N rates and cropping systems affected the C:N ratio of cauliflower leaves resulting in a progressive decrease from N0 to N3 where cauliflower was intercropped. The C:N ratio of cauliflower sole-cropped was not affected by N rates, but showed higher values compared to the intercropped cauliflower in N3.

The C:N ratio of clover aboveground biomass was unaffected by treatments and was on average higher than the C:N ratio of cauliflower leaves (Table 6). The N rates significantly affected the C:N ratio of clover roots. With N3, clover roots had a C:N ratio lower than in N0 and N1, while in N2, no differences were recorded.

3.4. Effects on growth and N uptake of lettuce

The growth and N content of lettuce was affected by both N rates and cropping system applied in 2015, as well as by their interaction (Table 7). Regardless of the preceding cropping system, the lowest growth and N content of lettuce was recorded in N0 plots. The effects of

Table 2
Cauliflower dry weight as affected by nitrogen rates (N), cropping systems (C.S.) and their interaction (N X C.S.). Data are means of the three replicates or grand means over N and C.S. Small letters indicate differences within columns; capital letters indicate differences within rows. Significance codes: "*" = $p \leq 0.05$, "·" = $p \leq 0.1$, "n.s." = not significant.

	Dry biomass (g m^{-2})						
Treatment	Roots	Stem	Curd	Leaves		Total	
N				Sole-cropped	Intercropped	Sole-cropped	Intercropped
N0	84.43	17.88	169.54 b	650.73 a A	313.95 d B	1039.78 a A	690.51 c B
N1	82.97	15.14	199.79 ab	524.37 a B	705.32 a A	978.69 a A	1105.66 a A
N2	90.70	19.73	236.84 a	556.08 a A	541.98 b A	1114.24 a A	950.55 b A
N3	82.85	20.44	182.59 b	671.07 a A	385.52 c B	1134.94 a A	730.62 c B
C.S.							
Sole-cropped	97.24 a	156.07 a	213.04 a				
Intercropped	73.24 b	128.06 b	181.34 b				
Significance							
N	n.s.	n.s.	*	n.s.		n.s.	
C.S.	·	*	*	*		*	
N X C.S.	n.s.	n.s.	n.s.	*		*	

Table 3
Cauliflower nitrogen content as affected by nitrogen rates (N), cropping systems (C.S.) and their interaction (N X C.S.). Data are means of the three replicates or grand means over N and C.S. Small letters indicate differences within columns; capital letters indicate differences within rows. Significance codes: “**” = $p \le 0.01$, “*” = $p \le 0.05$, “·” = $p \le 0.1$, “n.s.”= not significant.

	Nitrogen Content g m^{-2}						
Treatment	Roots	Stem	Curd	Leaves		Total	
N				Sole-cropped	Intercropped	Sole-cropped	Intercropped
N0	1.40	1.69	6.77 c	22.59 a A	11.65 c B	32.14 a A	21.82 b B
N1	1.56	1.89	7.89 bc	22.08 a A	27.79 a A	34.92 a A	37.65 a A
N2	1.84	1.97	11.51 a	21.58 a A	26.51 ab A	38.38 a A	40.38 a A
N3	2.04	2.04	8.97 b	22.46 a A	19.53 b B	37.09 a A	30.99 ab A
C.S.							
Sole-cropped	1.94 a	2.19 a	9.33				
Intercropped	1.47 b	1.61 b	8.25				
Significance							
N	n.s.	n.s.	**	n.s.		*	
C.S.	·	*	n.s.	n.s.		·	
N X C.S.	n.s.	n.s.	n.s.	**		*	

Table 4
Cauliflower leaf N concentration and soil mineral N (nitrate-N plus ammonium-N) after curd harvest as affected nitrogen rates (N), cropping systems (C.S.) and their interaction (N X C.S.). Data are means of the three replicates or grand means over N and C.S. Small letters indicate differences within columns; capital letters indicate differences within rows. Significance codes: “***” = $p \le 0.001$, “*” = $p \le 0.05$, “· “= $p \le 0.1$, “n.s.”= not significant.

Treatment	Leaves N%	Soil Mineral N - October 2015 (mg kg^{-1})		Soil Mineral N - May 2016 (mg kg^{-1})
N		Sole-cropped	Intercropped	
N0	4.18 b	2.12 b A	2.9 b A	2.87
N1	4.57 ab	2.84 ab A	2.92 b A	3.25
N2	5.05 ab	3.15 ab A	2.96 b A	5.29
N3	5.47 a	9.33 a B	16.18 a A	5.48
C.S.				
Sole-cropped	4.78			4.07
Intercropped	5.04			4.38
Significance				
N	·	***		n.s.
C.S.	n.s.	*		n.s.
N X C.S.	n.s.	*		n.s.

the preceding cropping system on lettuce growth and N content depended on the level of 2015 N supply in N1 and N2 plots, but not in N0 and N3. The intercropping with clover resulted in higher lettuce yield and N content (Table 7).

Table 6
Carbon:Nitrogen (C:N) ratio of cauliflower and clover as affected by N rates (N), cropping systems (C.S.) and their interaction (N X C.S.). Data are means of the three replicates or grand means over N and C.S. Small letters indicate differences within columns; capital letters indicate differences within rows. Significance codes: “*” = $p \le 0.05$, “· “= $p \le 0.1$, “n.s.”= not significant.

	Cauliflower C:N				Clover C:N	
Treatment	Roots	Stem	Leaves		Roots	Leaves
N			Sole-cropped	Intercropped		
N0	23.81 a	31.00	11.27 a A	10.91 a A	17.63 a	22.18
N1	20.87 a	34.95	9.93 a A	10.29 ab A	17.71 a	25.95
N2	19.25 ab	33.08	10.26 a A	8.75 ab A	15.22 ab	20.89
N3	15.04 b	29.15	11.71 a A	8.09 b B	15.01 b	17.90
C.S.						
Sole-cropped	20.14	30.51				
Intercropped	19.34	33.59				
Significance						
N	*	n.s.	n.s.		*	n.s.
C.S.	n.s.	n.s.	*		n.s.	n.s.
N X C.S.	n.s.	n.s.	·		n.s.	n.s.

4. Discussion

In our study, the clover has not provided additional N to the intercropped cauliflower and it adversely affected both its growth and N uptake if no N fertilizer was supplied. This suggests either a low atmospheric N fixation by rhizobia bacteria associated with clover roots and/or that a low transfer of N from atmospheric N fixation from clover

Table 5
Dry weight and N content of crop residues left on the soil after harvest as affected by N rates (N), cropping systems (C.S.) and their interaction (N X C.S.). In the “intercropped” columns, data are referred to the DW and N content of cauliflower and clover residues pooled together. Data are means of the three replicates or grand means over N and C.S. Small letters indicate differences within columns; capital letters indicate differences within rows. Significance codes: “*” = $p \le 0.05$, “· “= $p \le 0.1$, “n.s.”= not significant.

	Dry biomass g m^{-2}		Nitrogen g m^{-2}	
Treatment	Sole-cropped (Cauliflower)	Intercropped (Cauliflower + Clover)	Sole-cropped (Cauliflower)	Intercropped (Cauliflower + Clover)
N				
N0	861.57 a A	896.46 ab A	25.76 a A	21.99 b A
N1	765.73 a B	1287.23 a A	25.88 a B	36.62 a A
N2	852.67 a A	913.52 ab A	26.11 a B	32.89 ab A
N3	935.51 a A	774.08 b A	27.49 a A	27.13 ab A
Significance				
N	n.s.		n.s.	
C.S	·		·	
N X C.S.	*		*	

Table 7
Lettuce biomass and N content as affected by residual fertility of N rates (N), cropping systems (C.S.) and their interaction (N X C.S.). Data are means of the three replicates or grand means over N and C.S. Small letters indicate differences within columns; capital letters indicate differences within rows. Significance codes: "**" = p ≤ 0.01, "*" = p ≤ 0.05, "· " = p ≤ 0.1.

	Lettuce total biomass (DW g m^{-2})		Lettuce total N content (g m^{-2})	
Treatment	Sole-cropped	Intercropped	Sole-cropped	Intercropped
N				
N0	171.29 b A	222.05 b A	6.52 b A	8.72 b A
N1	233.80 a B	300.60 a A	8.28 ab B	11.70 ab A
N2	236.30 a B	305.00 a A	9.21 a B	12.62 a A
N3	236.53 a A	235.47 ab A	9.05 a A	9.29 ab A
Significance				
N	*		*	
C.S.	**		**	
N X C.S.	·		·	

to the intercropped cauliflower plants. It might also be possible that clover has negatively affected cauliflower growth due to competition for light during the vegetative stages (Thorsted et al., 2006; den Hollander et al., 2007), however, the fact that such competition was not present when N fertilizer at the two rates N1 and N2 was provided, suggests competition for N was the main reason for growth depression of intercropped cauliflower at N0. The N uptake by clover plants (Table 1, supplementary material) in N0 plots was in fact approximately one third of that of cauliflower (Table 3), whilst it ranged from 9 to 18% in the plots where N fertilizers were supplied. It should be remembered that the clover was sown just prior to cauliflower transplanting and that legumes depend on soil inorganic N during their early growth stages after germination, a fact that might have led to strong competition between the two species (Xie et al., 2017). Our findings are in accordance with studies involving bean (Yildirim and Guvenc, 2005), and other brassica species as intercrop (Bavec et al., 2012). The competition for vegetative growth has not affected the reproductive growth (the curd) of cauliflowers, a fact suggesting that competition was not present during the whole cauliflower crop cycle or that the intercropped cauliflower has allocated relatively more biomass into the curd to safeguard its reproductive growth (Burns, 1996). A similar behavior was observed by Westarp et al. (2004), who found that cauliflower subject to water stress preferentially partitions resources to the cauliflower curd, at the expense of leaves.

Our study has also shown the response of cauliflower to increasing N supply. This crop is known to be highly demanding in terms of N uptake. Under our experimental conditions the marketable yields ranged between 25 and 47 t ha^{-1}, while the N uptake rates (estimated by N content) ranged from 270 to 395 kg ha^{-1}, depending on the N rate and the presence or absence of the intercropped species. These amounts are higher than those observed in Everaarts (1993), Everaarts et al. (1996) and Di Gioia and Santamaria (2009), but similar to Vázquez et al. (2010) (250–498 kg N ha^{-1}). We speculate that the pre-existing soil fertility provided significant amounts of N for root uptake in addition to the fertilizer N, to the point that the N1 rate (75 kg N ha^{-1}) maximized N uptake, plant growth and yields. Regardless of the rate of N supply, most absorbed N was allocated to the leaves and to the other organs left on top of the soil as residues at the end of the cycle while the N amount removed with the curds ranged from 67 to 115 kg N^{-1} (Table 3).

The highest N rates used in our study (N3), besides being slightly detrimental to curd yields, significantly increased the residual mineral N in the soil after harvest, which might have been prone to leaching, due to the rainfall during October 2015 to May 2016 (Fig. 1). Under the highest N regime (N3), this effect has not been counteracted by the presence of an intercropped legume.

In our study, cauliflower residues contained 70–75% of the total N amount taken up by the crop (150–300 kg N ha^{-1}), while the clover residues had ~30-75 kg N ha^{-1}. This data is in line with literature (den Hollander et al., 2007). It is known that the timing of residue incorporation affects their decomposition and future nutrient release. Chen et al. (2014) found that crop residue decay rates are generally slower when they are left on the soil surface than when incorporated in the soil. In our study, the fact that the crop residues were initially left on top of the soil and then incorporated in the following spring might have limited their degradation during the winter and allowed a more efficient nutrient recycling (Mitchell et al., 2000).

Both quality and quantity of the crop residues are known to affect their decomposition and N release. The decay rate of crop residues is at least in part inversely related to C:N ratio (Vigil and Kissel, 1991) and a high C:N ratio is known to cause, at least initially, some N immobilization (Lemtiri et al., 2016). Similar to other findings (Chaves et al., 2007), crop residues of cauliflower had C:N ratios ranging from 8 to 12 in the leaves and from 15 to 35 in roots and stem, suggesting a faster decay rate of the leaves compared to roots and stems. In the annual clover, the C:N ratio ranged from 15 to 18 in roots and from 18 to 26 in leaves.

Even though intercropping annual clover with cauliflower did not cause any increase in the curd yields, the benefit of the intercropping system has been observed to the subsequent crop. Clover enhanced the total biomass and amount of N of residues from N1 and N2 plots (Table 5) and this resulted in a promoted growth and N uptake by lettuce plants in the subsequent cropping cycle (Table 7). The effects of the preceding cauliflower-clover crop on biomass and N uptake of lettuce could also be due to the general improved physical conditions of the soil left by the presence of clover (McVay et al., 1989). Indeed, the presence of a legume species has been shown to reduce soil strength, enhancing soil porosity and water retention (Rochester et al., 2001), thus allowing an easier roots establishment and development of the succeeding crop.

5. Conclusions

When cauliflower was cultivated alone, the growth and N uptake were unaffected by N fertilizer rates, whereas the highest yields were obtained with the N-fertilizer rate equal to 75 kg N ha^{-1}. This N rate also maximized N uptake, plant growth, and yields when cauliflower was intercropped with clover. Intercropping a N-fixing species like clover did not affect either the cauliflower marketable yield or the N uptake when the crop was fertilized with N. However, where no N was supplied, growth and N uptake by intercropped cauliflower was hampered by the presence of clover.

The benefit of intercropping clover with cauliflower was observed on the growth and N uptake by the lettuce crop that followed cauliflower. The higher amount of N present in the residues left after by both cauliflower and clover in the intercropped plots fertilized with 75–150 kg N ha^{-1} caused the highest growth and N uptake of lettuce plants grown in succession. In conclusion, although the presence of clover did not reduce the N fertilizer needs of the intercropped cauliflower, it contributed to enhancing the soil N availability and yields of the succeeding crop.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.scienta.2018.11.020.

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