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A comprehensive study of planting density and nitrogen fertilization effect on dual-purpose hemp (*Cannabis sativa* L.) cultivation

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ABSTRACT

Harvesting hemp (*Cannabis sativa* L.) for both stems and seeds is now a common practice in Europe while crop management strategies for dual-purpose hemp cultivation have not been properly addressed so far. In the present study, the effects of planting density and nitrogen fertilization on hemp stem and seed yields were tested with the cultivars Futura 75 and/or Bialobrzeskie in eight contrasting environments (Italy in 2013; Italy and Latvia in 2014; Italy (two sites), Latvia, the Czech Republic, and France in 2015). Stem yield ranged between 1.3 and 22.3 Mg ha^{-1}. The effects of planting density and nitrogen fertilization on stem yield did not interact significantly with each other, or with cultivar and harvest time. Increasing planting density from 30 to 120 plants m^{-2} and increasing nitrogen fertilization rate from 0 to 60 kg N ha^{-1} increased stem yield by 29% and 32%, respectively. Further increase in planting density and nitrogen fertilization did not result in a significant increase in stem yield. Seed yield ranged from 0.3 to 2.1 Mg ha^{-1}. The seed yield was not affected significantly by planting density between 30 and 240 plants m^{-2}. Although the seed yield showed an increasing trend with increasing nitrogen fertilization, the effects of nitrogen fertilization on seed yield were not statistically significant.

To grow hemp as a dual-purpose crop it is recommended to plant 90–150 plants m^{-2} across all tested environments. Nitrogen fertilization rate at 60 kg N ha^{-1} was generally sufficient in the tested environments whereas further optimization of nitrogen fertilization requires accurate assessment of plant nitrogen status. To facilitate assessing plant nutritional status, a critical nitrogen dilution curve was determined for hemp and a practical method to determine nitrogen nutritional status was discussed.

1. Introduction

Hemp (*Cannabis sativa* L.) is resurging as an ideal multipurpose crop worldwide (Amaducci et al., 2015; Aubin et al., 2016; Faux et al., 2013; Bertoli et al., 2010). For the first time, it was cultivated in Europe on more than 33,000 ha in 2016 mainly as a dual-purpose crop where stems and seeds were harvested simultaneously (Carus 2017). However, hemp was traditionally a fibre crop and most past research focused on this purpose (Westerhuis et al., 2009; Amaducci et al., 2008a, 2002a; Struik et al., 2000; Van der Werf et al., 1996). Very limited information is available on growing dual-purpose hemp (Amaducci et al., 2015). In the frame of the EC funded project Multihemp (www.multihemp.eu), extensive experiments have been carried with the aim of providing novel information to support dual-purpose hemp cultivation in Europe. Aspects related to cultivar choice for dual-purpose hemp cultivation have been recently published by Tang et al. (2016). The present study focuses on the effect of the two main agronomic practices affecting the performance of dual-purpose hemp: planting density and nitrogen fertilization.

The effects of planting density and nitrogen fertilization on both stem and seed yields have not been properly addressed so far. Previous researches indicate that planting density has little effect on stem yield, but plants grown at high density are shorter and thinner than those grown at low density (Amaducci et al., 2002b; Struik et al., 2000).

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Slender stems are desirable for fibre hemp production because they produce more long fibre (Westerhuis et al., 2009) and require less energy for their mechanical processing (Khan et al., 2010). Thus, a high planting density is generally used, ranging from 90 plants m^{-2} to 350 plants m^{-2} (Martinov et al., 1996; Starcevic, 1996), to achieve required fibre quantity and quality. On the other hand, a low planting density, ranging from 30 plants m^{-2} to 75 plants m^{-2}, is recommended for producing hemp seeds (Amaducci and Gusovius, 2010 and references therein). Optimal planting density has not been researched for growing hemp as a dual-purpose crop.

The effect of nitrogen fertilization on stem yield varies in literature. For relatively low fertility conditions, Amaducci et al. (2002b) reported that stem yield increased by 20 kg kg^{-1} N. Finnan and Burke (2013) reported a very high stem yield increase with increasing fertilization from 0 up to 120 kg N ha^{-1} (as high as 60 kg kg^{-1} N). In contrast, the yield response of hemp to nitrogen fertilization was found negligible when soil fertility was high (Prade et al., 2011; Struik et al., 2000). Few studies have been conducted considering the response of seed yield to nitrogen fertilization. Aubin et al. (2015) and Marija et al. (2011) reported that both stem and seed yields were positively related to nitrogen fertilization. Vera et al. (2010, 2004) reported that hemp seed yield increased progressively with increasing nitrogen availability until a high fertilization rate, ranging from 99 kg N ha^{-1} to 198 kg N ha^{-1} depending on growing conditions. Given the wide range of the results regarding stem and seed yields in response to nitrogen fertilization and the large variation of soil nitrogen availability, it is a challenge for farmers to optimize fertilization rate and to maximize economic return.

Nitrogen fertilization affects crop yield mainly through its effect on plant nitrogen status (Sadras and Lemaire, 2014). When nitrogen supply is deficient, aboveground biomass yield (W) increases with increasing nitrogen uptake until a critical nitrogen concentration (N_{critical}) has been reached; further increasing nitrogen uptake has little impact on increasing W (Lemaire and Meynard, 1997). In general, the N_{critical} decreases exponentially with increasing W during plant growth, which is called the N_{critical} dilution curve (Greenwood et al., 1991, 1990). Although the N_{critical} dilution curve varies among species, it remains fairly consistent at different environmental growth conditions (Lemaire and Gastal, 2009). Therefore, the N_{critical} dilution curve has been used to determine the nitrogen status for many crops, including rice (*Oryza sativa* L.; Ata-Ul-Karim et al., 2013; Sheehy et al., 1998), maize (*Zea mays* L.; Ziadi et al., 2010), oilseed rape (*Brassica napus* L.; Colnenne et al., 1998) and linseed (*Linum usitatissimum* L.; Flénet et al., 2006). Estimating a N_{critical} dilution curve for hemp would be useful to optimize its nitrogen fertilization.

The objective of this study was to assess the effects of planting density and nitrogen fertilization across a wide range of environments to support dual-purpose hemp cultivation in Europe. First, the effects of planting density and nitrogen fertilization on hemp stem and seed yields were investigated. Second, the characteristics of hemp's nitrogen demand were analysed and a critical nitrogen dilution curve was assessed for hemp.

2. Materials and methods

2.1. Experimental locations and field layout

Field experiments were carried out at five locations in Europe: Piacenza-IT (Piacenza, Italy), Budrio-IT (Budrio, Italy), FR (La Trugalle, France), CZ (Sumperk, the Czech Republic) and LV (Vilani, Latvia) from 2013 to 2015 (Table 1). Latitude difference between the most northern (LV) and the most southern (Budrio-IT) location was 12°, which corresponds to 2 h of maximum day-length. Average temperature between May and October (during the hemp growing season) ranged from 14.9 °C (LV) to 21.8 °C (Piacenza-IT); total precipitation ranged from 212 mm (CZ) to 297 mm (FR). The most southern locations, Piacenza-IT and Budrio-IT, were hot and dry in the summer while the other three locations were cool and humid (Supplementary Fig. 1).

Planting density was varied from 30 plants m^{-2} to 240 plants m^{-2}; nitrogen fertilization range was from 0 to 120 kg N ha^{-1}. The planting density × nitrogen fertilization interaction was tested with the cultivar Futura 75 in Piacenza-IT in 2013. The planting density × cultivar and nitrogen fertilization × cultivar interactions were tested with the cultivars Futura 75 and Bialobrzeskie in Piacenza-IT and LV in 2014. As the effects of planting density and nitrogen fertilization on stem and seed yields did not interact with each other in 2013 and did not interact with cultivar in 2014 (see Results section), the effects of planting density and nitrogen fertilization were tested separately in 2015 for the cultivar Futura 75 at all five locations (Piacenza-IT, Budrio-IT, FR, CZ and LV).

The main factors (i.e., planting density, nitrogen fertilization and cultivar) were tested in a randomized complete block design with four replicates. Single plot size was 42 m^2 when only two harvests were scheduled, or 60 m^2 when multiple harvests were scheduled for a detailed growth analysis. Sowings were carried out as soon as the soil was accessible and average daily temperature rose above 8–10 °C. Sowing dates spanned from the earliest on 7 April 2014 in Piacenza-IT to the latest on 14 May 2015 in Budrio-IT (Supplementary Table 1). Seeds were drilled at 3–4 cm depth using experimental-plot sowing machines. The distance between rows varied between 15 cm and 25 cm, depending on the sowing machine used at each location. Seed rates were calculated based on the target density considering weight of 1000 seeds and results of seed germination tests. In Piacenza-IT and Budrio-IT, densities of 30 plants m^{-2} and 60 plants m^{-2} were obtained by sowing seeds in excess (90 plants m^{-2}) and hand-thinning to target density after emergence. Hand-thinning was conducted carefully so that any impact (e.g. increase of soil compaction due to worker's footprint) on plant growth would be minimized. Nitrogen fertilizer was distributed at sowing or immediately after emergence. Irrigation was only applied in Piacenza-IT; in total 120 mm, 60 mm and 155 mm of water was provided with a travelling sprinkler in 2013, 2014 and 2015, respectively.

2.2. Assessing crop development and yields

Seedling emergence was monitored in the plots with 120 plants m^{-2} by counting the emerged plants in one row over a length of 1 m or in two rows over a length of 50 cm, every two days from the appearance of the first plant until full emergence. Date of emergence was set when 50% of final seedlings had emerged. Flowering was monitored on 20 representative plants that were selected and labelled before flowering. A single plant was considered at the full flowering stage when its top appeared as a compact (i.e., with very short internodes) inflorescence with visible stigmata. The full flowering stage of a plot was set when 50% of the monitored plants had reached full flowering.

In each plot, harvest was carried out at least twice: at full flowering (H1) and at seed maturity (H2). At each harvest, all plants in an area of 4 m^2 were cut just above the soil level. Fresh weight of all harvested plants was assessed immediately and the number of plants in the first harvested 1 m^2 was counted. Among the plants of this first 1 m^2, 20 representative plants were sampled. A subsample of 10 plants was dried at 75 °C until constant weight to assess dry matter content. On the remaining 10 plants, stem diameter (at 10 cm from stem base), plant height and proportion of stem, leaf and seed (H2) in the above ground biomass (after oven drying) were assessed. W (aboveground biomass yield) was calculated as the product of fresh weight and dry matter content. The yields of stem (W_{stem}), leaf (W_{leaf}) and seed (W_{seed}) were estimated as the product of W and the corresponding proportions.

In addition to H1 and H2, periodic samplings were carried out on 1 m^2 area in Piacenza-IT (2014 and 2015) and in Burdio-IT (2015), in total 4–5 times. At each sampling date, all plants in an area of 1 m^2 were cut just above the soil level using pruning scissors. Plant density, plant height, stem diameter, W, W_{stem}, W_{leaf} and W_{seed} (when present) were assessed following the same procedure described for H1 and H2.

Table 1
List of locations, soil characteristics and field managements.

Year	Location[a]	Geographical coordinates	Altitude (m asl)	Soil texture[b]	Soil total N (%)[b]	Soil organic matter content (%)[b]	Cultivars	Management combination[c]	Harvesting/sampling
2013	Piacenza-IT	45N;10E	60	Silty clay loam	0.18	2.8	Futura 75	D30; N(0–50-100) D60; N(0–50-100) D120; N(0–50-100) D240; N(0–50-100)	Full flowering and seed maturity
2014	Piacenza-IT	45N;10E	60	Silty clay loam	0.14	2.2	Futura 75 Bialobrzeskie	D60; N60 D120; N60 D240; N(0–30-60-120)	Full flowering Growth analysis
	LV	57N;27E	64	Sandy clay	NA	11.0	Futura 75 Bialobrzeskie	D60; N60 D120; N60 D240; N(0–30-60-120)	Full flowering
2015	Piacenza-IT	45N;10E	60	Silty clay loam	0.14	2.6	Futura 75	D30; N60 D60; N60 D120; N(0–30-60-120) D240; N60	Full flowering and seed maturity Growth analysis
	Budrio-IT	45N;12E	26	Silty clay loam	0.09	3.4	Futura 75	D30; N60 D60; N60 D120; N(0–30-60-120) D240; N60	Full flowering and seed maturity Growth analysis
	FR	48N;0E	67	Sandy loam	0.11	1.8	Futura 75	D30; N60 D60; N60 D120; N(0–30-60-120) D240; N60	Full flowering and seed maturity
	CZ	50N;17E	319	Loam	0.14	NA	Futura 75	D30; N60 D60; N60 D120; N(0–30-60-120) D240; N60	Full flowering and seed maturity
	LV	57N;27E	64	Sandy clay	0.12	6.5	Futura 75	D30; N60 D60; N60 D120; N(0–30-60-120) D240; N60	Full flowering

NA: data not available.
[a] The location names are abbreviated as: Piacenza-IT (Piacenza, Italy); Budrio-IT (Budrio, Italy); FR (La Trugalle, France), CZ (Sumperk, the Czech Republic) and LV (Vilani, Latvia).
[b] Soil sampling depth was 0–40 cm at Piacenza-IT, Budrio-IT and CZ; 0–30 cm at FR and 0–35 cm at LV.
[c] D and N stands for planting density (plants m^{-2}) and nitrogen fertilization (kg N ha^{-1}) factors, respectively.

Total nitrogen concentration in stem (N_{stem}), leaf (N_{leaf}) and seed (N_{seed}, if present) was analysed for each nitrogen treatment plot using a *CN* analyser (Vario Max CN Analyzer; Elementar Americas, Inc., Hanau, Germany). In Piacenza-IT (2014 and 2015), the interception of photosynthetically active radiation (PAR) by the canopy was measured before each sampling with a ceptometer (Decagon Devices, Inc., Pullman, Washington, USA); leaf area index (*LAI*) was calculated as the product of W_{leaf} and specific leaf area (*SLA*). The *SLA* was determined as the ratio of leaf area and dry weight of all leaves of two representative plants per plot. The leaf area was determined from scanned pictures using ImageJ (version 1.49; https://imagej.nih.gov/). In Piacenza-IT in 2015, *SPAD* (SPAD-502, Minolta, Japan) measurements were taken at each harvesting time on 3 marked representative plants per plot.

2.3. Nitrogen demand analysis

When plant nitrogen is limiting, *W* generally increases with increasing nitrogen concentration (*N*) until a $N_{critical}$ (critical nitrogen concentration) has been reached; above the $N_{critical}$, *W* is little dependent of *N* indicating that nitrogen is in excess. The $N_{critical}$ (%) decreases with increasing *W* (Mg ha^{-1}) during the vegetative growth period. This relationship can be described by a negative power function (Greenwood et al., 1991, 1990; Lemaire et al., 2008a):

$$N_{critical} = \begin{cases} aW^{-b} & W \geq W_{threshold} \\ N_{constant} & W < W_{threshold} \end{cases} \tag{1}$$

where *a* represents the value of $N_{critical}$ for $W = 1$ Mg ha^{-1}; *b* represents the ratio between the relative decline in $N_{critical}$ and the relative crop growth rate. $W_{threshold}$ (Mg ha^{-1}) represents the minimum *W* for which the relationship between $N_{critical}$ and *W* can be described using Eqn. (1); $N_{constant}$ (%) means the *N* when $W < W_{threshold}$. Multiplying Eq. (1) by *W* (Mg ha^{-1}) gives the critical curve for above ground nitrogen uptake ($N_{uptake,cri}$; kg N ha^{-1}):

$$N_{uptake,cri} = \begin{cases} 10aW^{1-b} & W \geq W_{threshold} \\ 10N_{constant}W & W < W_{threshold} \end{cases} \tag{2}$$

The ratio of *N*: $N_{critical}$ or N_{uptake}: $N_{uptake,cri}$, called nitrogen nutrition index (*NNI*), can be used to diagnose plant nitrogen status. $NNI < 1$ indicates nitrogen is limiting while $NNI \geq 1$ indicates nitrogen is sufficient.

To determine a $N_{critical}$ dilution curve for hemp, the relationship between *W* and N_{uptake} in Piacenza-IT (2014 and 2015) and in Budrio-IT (2015) was assessed for solving the coefficients *a*, *b*, $W_{threshold}$ and $N_{constant}$ in Eq. (1) and Eq. (2). The methodology proposed by Justes et al. (1994) was adopted. Specifically, for each sampling date when both nitrogen limiting and non-limiting treatments occurred, a value of $N_{uptake,cri}$ was identified at the intersection of an oblique line (positive linear regression between N_{uptake} and *W*) and a vertical line (relative to the average value of *W* in non-limiting nitrogen conditions) in the plot of N_{uptake} against *W* (see Fig. 7A in Results section), using the GAUSS method in PROC NLIN of SAS (SAS Institute Inc., Cary, North Carolina, USA). The nitrogen limiting and non-limiting treatments were identified by analysis of variance (SPSS statistics 22.0, SPSS, Chicago, Illinois, USA). A condition of nitrogen limitation occurs whenever *W* increased significantly ($P < 0.05$) with an increase in N_{uptake}. A condition of non-limiting nitrogen condition occurs whenever *W* remained unchanged with an increase in N_{uptake}. All identified $N_{uptake,cri} - W$ points were fitted to Eq. (2) for the value of *a*, *b*, $W_{threshold}$ and $N_{constant}$.

Under changing nitrogen supply in the field, rather than instant nitrogen status, the plant biomass production is the result of the nitrogen nutrition status during the whole growth season. Therefore, nitrogen deficiency duration and intensity were integrated into an index of crop nitrogen status, NNI_{int} (Jeuffroy and Bouchard, 1999). The NNI_{int} was calculated as: $1/n\ \Sigma n_i NNI_i$, where n means the growth duration, expressed in degree-days (°Cd) in this study; n_i means the representing duration of ith sampling; NNI_i means the NNI at ith sampling. The degree-days was calculated as the time integral of T_{ave} - T_b, with T_{ave} being the daily average temperature and T_b = 1 °C (Lisson et al., 2000; Van der Werf et al., 1995a).

2.4. Statistical analysis

Mixed models were used to assess the effects of planting density (D) and nitrogen fertilization (N) on biomass production and biometric traits using SPSS statistics 22.0 (SPSS, Chicago, Illinois, USA). Harvests at different times were conducted in the same plot; therefore, harvesting time (H) was considered as a repeated factor. Location, year and block were considered as random factors (Blouin et al., 2011). If the effect of one factor on a dependent variable was significant, multiple comparison was performed using the Bonferroni method. The data collected in 2013 was analysed to assess the D × N effect. The data collected in 2014 was analysed to assess the D and N effects in association with cultivar (G). The overall D and N effects were analysed by pooling the data collected in 2014 and 2015 at different locations.

3. Results

Seedling emergence was attained at 75 °Cd–112 °Cd after sowing and full flowering at 1150 °Cd–1982 °Cd, depending on growing location and cultivar (Supplementary Table 1). Harvest was carried out on average at 94 °Cd and 878 °Cd after full flowering for H1 (full flowering) and H2 (seed maturity), respectively.

3.1. The effects of planting density and nitrogen fertilization on stem and seed yields

W_{stem} (stem yield) ranged from 1.3 Mg ha^{-1} until 10.6 Mg ha^{-1}, from 7.8 Mg ha^{-1} until 9.6 Mg ha^{-1}, from 2.8 Mg ha^{-1} until 5.2 Mg ha^{-1}, from 8.1 Mg ha^{-1} until 18.8 Mg ha^{-1} and from 13.7 Mg ha^{-1} until 22.3 Mg ha^{-1} in Piacenza-IT (2013, 2014 and 2015), Budrio-IT, FR, CZ and LV (2014 and 2015), respectively. The effect of planting density and nitrogen fertilization on W_{stem} did not interact significantly with each other (Supplementary Table 2), or with the effects of cultivar (Supplementary Table 3) and harvest time (Table 2). The W_{stem} was increased significantly with increasing planting density and nitrogen fertilization rate (Table 2). Increasing planting density from 30 plants m^{-2} to 120 plants m^{-2} resulted in an overall increase in W_{stem} by 29% while the difference in W_{stem} was not significant between 120 plants m^{-2} and 240 plants m^{-2} (Table 2). Increasing nitrogen fertilization rate from 0 to 60 kg N ha^{-1} resulted in an overall increase in W_{stem} by 32% while the difference in W_{stem} was not significant between 60 kg N ha^{-1} and 120 kg N ha^{-1}. The effect of planting density and nitrogen fertilization on W_{stem} varied at specific environments. The variation was larger among nitrogen treatments than among planting densities (Fig. 1).

Plant height ranged from 55 cm (Piacenza-IT, 2014) to 312 cm (LV, 2015); stem diameter ranged from 2.6 mm (Piacenza-IT, 2014) to 11.4 mm (CZ, 2015). Increasing plant density from 30 plants m^{-2} to 240 plants m^{-2} resulted in overall decreases in plant height and stem diameter by 15% and 37%, respectively (Table 2). Increasing nitrogen fertilization rate from 0 to 120 kg N ha^{-1} resulted in overall increases in plant height and stem diameter by 22% and 20%, respectively.

W_{seed} (seed yield) range was 0.75–2.14 Mg ha^{-1}, 0.26–0.37 Mg ha^{-1}, 0.56–0.75 Mg ha^{-1} and 0.88–1.09 Mg ha^{-1} in Piacenza-IT (2013 and 2015), Budrio-IT, FR and CZ, respectively. W_{seed} was not determined in 2014 in both locations (i.e., Piacenza-IT and LV) nor in 2015 in LV. W_{seed} showed an increasing trend with an increase in nitrogen fertilization whereas the overall effects of both planting density and nitrogen fertilization on W_{seed} were not statistically significant (Table 2 and Fig. 2).

3.2. The effects of planting density and nitrogen fertilization on plant growth

Canopy closure was reached fast at high planting density and high nitrogen fertilization. In Piacenza-IT in 2015, light interception with 240 plants m^{-2} reached 90% at 732 °Cd after emergence that was significantly earlier than with 30 plants m^{-2} (1065 °Cd; Fig. 3A); 90% of light interception with 120 kg N ha^{-1} was reached at 700 °Cd after emergence that was 402 °Cd and 1072 °Cd earlier than with 30 kg N ha^{-1} and the unfertilised control treatment, respectively (Fig. 4A). The overall light extinction coefficient was 0.96. The earlier canopy closure was mainly a consequence of a significantly higher leaf area index (LAI) that was proportional to the level of planting density and nitrogen fertilization (Figs. 3 B and 4 B). After canopy closure, the LAI continued to increase until full flowering while light interception remained constant, slightly above 90%. The difference of LAI among planting density treatments progressively reduced after canopy closure while it remained significant among nitrogen fertilization treatments. In Piacenza-IT in 2015, no significant difference of LAI among planting densities was observed at full flowering while the LAI with the highest nitrogen fertilization level (6.4 m^2 m^{-2}) was about three times higher than that of the control treatment (2.3 m^2 m^{-2}). W (aboveground biomass yield) increased in accordance with LAI (Figs. 3 C and 4 C). The relationship between LAI and W was not affected by planting density or nitrogen fertilization (Figs. 3 D and 4 D).

Plant height and stem diameter increased exponentially with increasing W_{stem} (R^2 > 0.76; Fig. 5). This relationship was affected by planting density but it was independent from nitrogen fertilization. Considering the same stem yield level, plants cultivated at low planting densities were tallest and thickest.

3.3. Dynamics of nitrogen uptake and nitrogen concentration

N_{uptake} (above ground nitrogen uptake) and N (above ground nitrogen concentration) were proportional to the level of nitrogen fertilization. At the first sampling date in Piacenza-IT in 2015, N_{uptake} and N of the unfertilised control treatment were 34.8 kg N ha^{-1} and 4.3%, respectively. Nitrogen fertilization with 120 kg N ha^{-1} resulted in increases in N_{uptake} and N by 5.3 times and 1.9 times, respectively (Fig. 6A, B). During the growing season, N_{uptake} increased uniformly in the unfertilised control treatment until the end of flowering while the increase at increasing fertilization levels was more intense before canopy closure. The N_{uptake}: LAI ratio was higher with additional fertilization than with no fertilization (Fig. 6C). From the end of flowering to seed maturity, N_{uptake} was consistent in the unfertilised control treatment while it decreased slightly in the fertilized plots. N decreased moderately in the unfertilised control treatment and progressively more intense at increasing fertilization levels. In Piacenza-IT in 2015, significant differences in N among nitrogen treatments were present until full flowering (1970 °Cd). After full flowering, the N was identical among nitrogen treatments.

Considering the analysis of samples collected at seed maturity in Piacenza-IT in 2013–2015, N_{leaf}, N_{stem} and N_{seed} ranges were 1.5%–2.6%, 0.5%–0.7%, and 2.7%–4.0%, respectively (Table 3). Without fertilization N_{uptake} at seed maturity was 150.5 kg N ha^{-1}, 23.0 kg N ha^{-1}, and 74.2 kg N ha^{-1} in 2013, 2014, and 2015, respectively (Table 3). With respect to the unfertilised control treatment the N_{uptake} at the highest fertilization treatment (120 kg N ha^{-1}) increased by 57.7 kg N ha^{-1}, 94.3 kg N ha^{-1}, and 53.5 kg N ha^{-1} in 2013, 2014, and 2015, respectively. Nitrogen utilization efficiency at seed maturity ranged from 75.8 kg DM kg N^{-1} to 108.8 kg DM kgN^{-1}, independently

Table 2
The effect of planting density and nitrogen application on the biomass yields and plant biometrics. Data collected in 2014 and 2015 were pooled in the analysis using mixed models with location and year as random effects.

	Plant number (plants m^{-2})	Height (cm)	Diameter (mm)	Biomass yield (Mg ha^{-1})	Stem yield (Mg ha^{-1})	Leaf yield (Mg ha^{-1})	Seed yield (Mg ha^{-1})
Density (D)[a]							
D30	33 d[d]	217.2 c	9.4 a	10.0 c	7.7 c	1.6 a	0.8 a
D60	55 c	200.1 b	7.9 b	11.2 bc	9.0 b	1.7 a	0.7 a
D120	93 b	194.2 ab	7.1 c	12.3 ab	9.9 ab	1.8 a	0.7 a
D240	170 a	183.7 a	6.0 d	13.2 a	10.9 a	1.9 a	0.8 a
Nitrogen (N)[b]							
N0	88 a	177.3 c	6.8 c	9.4 c	7.5 c	1.4 d	0.6 a
N30	89 a	193.6 b	7.5 bc	11.4 b	9.2 b	1.6 c	0.8 a
N60	91 a	208.3 ab	7.9 ab	12.4 b	9.9 ab	1.9 b	0.8 a
N120	84 a	215.9 a	8.2 a	13.6 a	10.9 a	2.2 a	0.7 a
Harvest (H)[c]							
H1	88 a	192.9 a	7.5 a	11.2 a	8.9 b	2.0 a	NA[e]
H2	88 a	204.7 a	7.7 a	12.2 a	10.0 a	1.5 b	0.7
Statistics (*P* values)							
D	0.000	0.000	0.000	0.000	0.000	0.035	0.670
N	0.430	0.000	0.000	0.000	0.000	0.000	0.227
H	0.914	0.078	0.439	0.098	0.049	0.000	NA
D × H	0.016	0.860	0.982	0.379	0.565	0.024	NA
N × H	0.035	0.545	0.643	0.147	0.335	0.000	NA

[a] D30, D60, D120 and D240 stand for planting density 30 plants m^{-2}, 60 plants m^{-2},120 plants $m^{-2}$and 240 plants m^{-2}, respectively.
[b] N0, N30, N60 and N120 stand for nitrogen application rate 0 kg N ha^{-1}, 30 kg N ha^{-1}, 60 kg N $ha^{-1}$and 120 kg N ha^{-1}, respectively.
[c] H1 and H2 stand for harvesting at full flowering and seed maturity, respectively.
[d] Numbers followed by different letters under the same category are statistically different for $P < 0.05$(Bonferroni test).
[e] Not available.

of nitrogen treatments and growing years (Table 3).

3.4. The plant nitrogen nutrition status

None of the sampling dates in Piacenza-IT in 2014 includes both nitrogen limiting and non-limiting treatments. Among the sampling dates in Piacenza-IT and Budrio-IT in 2015, 10 sampling dates were identified for which both nitrogen limiting and non-limiting treatments were included. By assessing $N_{\text{uptake,cri}} - W$ points for each of the 10 sampling dates and fitting the $N_{\text{uptake,cri}} - W$ points to Eq. (2), the coefficients *a*, *b*, $W_{\text{threshold}}$ and N_{constant} were obtained. The $N_{\text{uptake,cri}}$ (kg N ha^{-1}) $-$ W (Mg ha^{-1}) curve is represented in Fig. 7A and its mathematical expression is as follows:

$$N_{\text{uptake,cri}} = \begin{cases} 32.6W^{0.62} & W \geq 0.78\ \mathbf{M}\text{gha}^{-1} \\ 36.0W & \boldsymbol{W} < 0.78\ \text{Mgha}^{-1} \end{cases} \tag{3}$$

Therefore, the N_{critical} (%) dilution curve can be expressed as:

$$N_{\text{critical}} = \begin{cases} 3.26W^{-0.38} & \boldsymbol{W} \geq 0.78\ \text{Mgha}^{-1} \\ 3.60 & W < 0.78\ \text{Mgha}^{-1} \end{cases} \tag{4}$$

The $N_{\text{uptake,cri}} - W$ curve separated accurately the nitrogen status of hemp crops in Piacenza-IT (2014 and 2015) and Budrio-IT (2015) (Fig. 7B). Nitrogen nutrition index (*NNI*), calculated as the ratio of *N*: N_{critical}, for each nitrogen treatment and sampling date in Piacenza-IT in 2015 is presented in Fig. 6D. The *NNI* of the unfertilised control treatment remained constant throughout the whole growing season at about 0.5. Nitrogen fertilization increased *NNI* at all sampling dates. During plant growth, the *NNI* with additional fertilization decreased gradually. At 30 kg N ha^{-1} and 60 kg N ha^{-1} the decrease of the *NNI* was limited to the period between the first and the second samplings whereas it decreased steadily throughout the whole growing season with 120 kg N ha^{-1}. Consequently, at flowering the difference of *NNI* among nitrogen treatments was reduced. NNI_{int}, calculated on the basis of nitrogen deficiency duration and intensity, positively correlated with

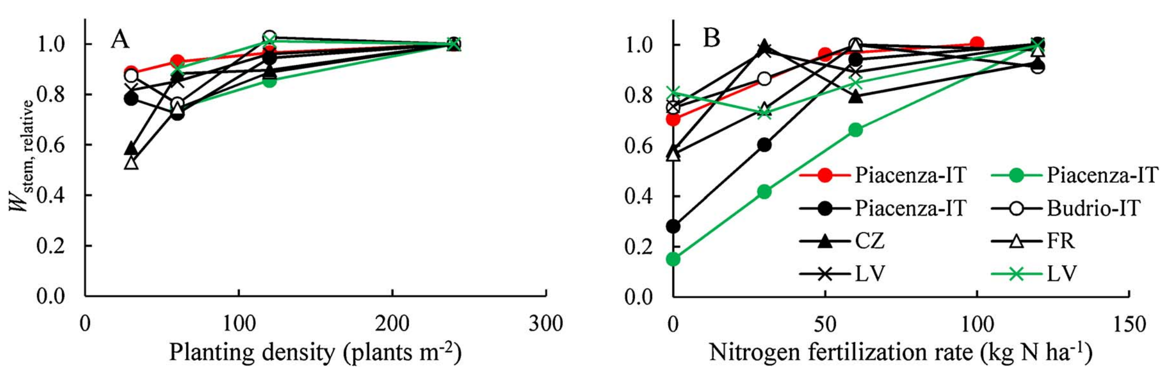

Fig. 1. The effect of planting density (A) and nitrogen fertilization (B) on relative stem yield ($W_{\text{stem,relative}}$) in different environments. The data presented was collected at full flowering in 2013 (red), 2014 (green) and 2015 (black). The location names are abbreviated as: Piacenza-IT (Piacenza, Italy); Budrio-IT (Budrio, Italy); FR (La Trugalle, France); CZ (Sumperk, the Czech Republic) and LV (Vilani, Latvia). The $W_{\text{stem,relative}}$ was calculated as the ratio of actual stem yield and the maximum stem yield at each location. In 2013, the maximum stem yield in Piacenza-IT was 8.2 Mg ha^{-1} and 8.7 Mg ha^{-1} across density and nitrogen treatments, respectively. In 2014, the maximum stem yield across density treatments in Piacenza-IT and LV was 5.6 Mg ha^{-1} and 19.2 Mg ha^{-1}, respectively; across nitrogen treatments, it was 8.5 Mg ha^{-1} and 22.3 Mg ha^{-1}, respectively. In 2015, the maximum stem yield across density treatments in Piacenza-IT, Budrio-IT, FR, CZ and LV was 7.9 Mg ha^{-1}, 9.5 Mg ha^{-1}, 5.6 Mg ha^{-1}, 15.2 Mg ha^{-1} and 16.8 Mg ha^{-1}, respectively; across nitrogen treatments, it was 7.9 Mg ha^{-1}, 9.5 Mg ha^{-1}, 5.0 Mg ha^{-1}, 15.9 Mg ha^{-1} and 18.1 Mg ha^{-1}, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

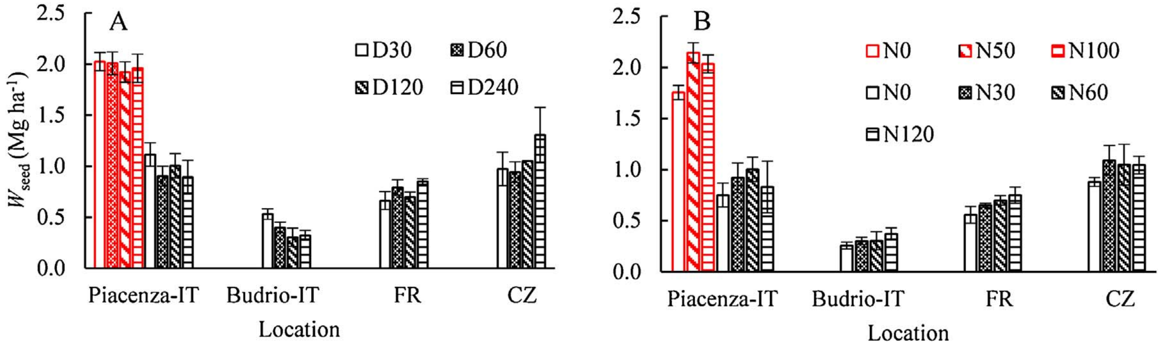

Fig. 2. The effect of planting density (A) and nitrogen fertilization (B) on seed yield (W_{seed}) in 2013 (red bars) and 2015 (black and white bars). The location names are abbreviated as: Piacenza-IT (Piacenza, Italy); Budrio-IT (Budrio, Italy); FR (La Trugalle, France) and CZ (Sumperk, the Czech Republic). Vertical bars indicate standard error. D30, D60, D120 and D240 stand for planting density 30 plants m^{-2}, 60 plants m^{-2},120 plants m^{-2}and 240 plants m^{-2}, respectively. N0, N30, N50, N60, N100 and N120 stand for nitrogen application rate 0 kg N ha^{-1}, 30 kg N ha^{-1}, 50 kg N ha^{-1}, 60 kg N ha^{-1},100 kg N ha^{-1}and 120 kg N ha^{-1}, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

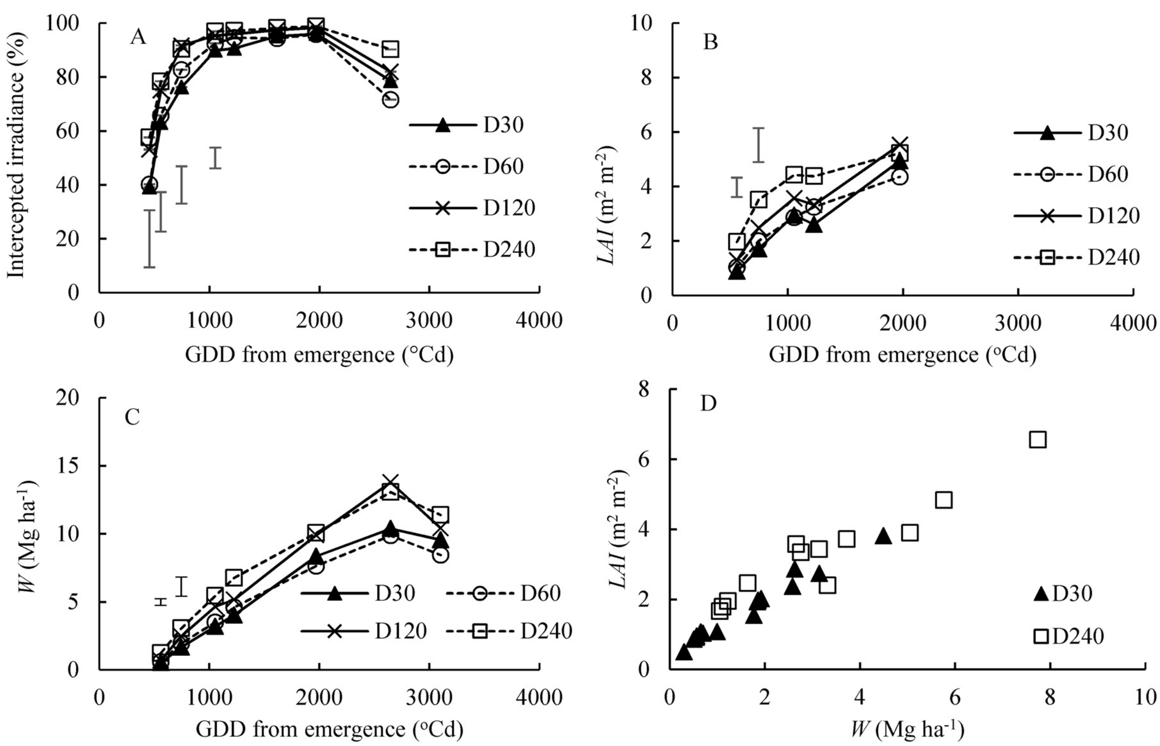

Fig. 3. The effect of planting density on growth. Panels A, B and C indicate the time course of light interception, leaf area index (*LAI*) and biomass yield (*W*), respectively. Panel D is the plot of *LAI* against *W* before flowering. Data presented was collected in the field in Piacenza, Italy in 2015. D30, D60, D120 and D240 stand for planting density 30 plants m^{-2}, 60 plants m^{-2}, 120 plants m^{-2} and 240 plants m^{-2}, respectively. Vertical bars in Panels A, B, C indicate the Bonferroni LSD for which the effect of planting density was significant at $P < 0.05$.

relative W ($W_{relative}$: calculated as the ratio of actual W and maximum W at the same sampling date) when the NNI_{int} was lower than 1. The relationship between $W_{relative}$ and NNI_{int} was independent of growth environments (Fig. 8).

4. Discussion

Growing hemp as a multi-purpose crop is gaining attention, particularly for producing both stems and seeds (Tang et al., 2016; Aubin et al., 2016; Faux et al., 2013). While numerous studies have been carried out to improve hemp cultivation for fibre production (e.g., Westerhuis et al., 2009; Struik et al., 2000; Amaducci et al., 2008a, 2002a; De Meijer et al., 1995), very limited information is available on the agronomy of dual-purpose (i.e., stems and seeds) hemp crops (Amaducci et al., 2015). In this paper, data obtained in eight environments (combinations of year and location) at five contrasting locations throughout Europe were analysed to study the effect on stem and seed yields of the main agronomic factors affecting hemp production: planting density and nitrogen fertilization. Considering that the effect of these two factors on hemp stem and seed yields did not interact with each other in 2013 in Piacenza-IT (Supplementary Table 2) and did not interact with cultivar in 2014 in Piacenza-IT and LV (Supplementary Table 3), which confirms results of previous research (Amaducci et al., 2008a, 2002a; Struik et al., 2000), results of planting density and nitrogen fertilization will be discussed separately.

4.1. The effects of planting density on stem and seed yields

In line with previous studies (Amaducci et al., 2008a, 2002a; Struik et al., 2000), increasing planting density from 30 plants m^{-2} to 240 plants m^{-2} had limited effect on W_{stem} (stem yield) (Table 2). The lack of response of W_{stem} across a wide density range is mainly a consequence of the high incidence of self-thinning at high planting density (Van der Werf et al., 1995b; Willey and Heath, 1969) and of the plastic

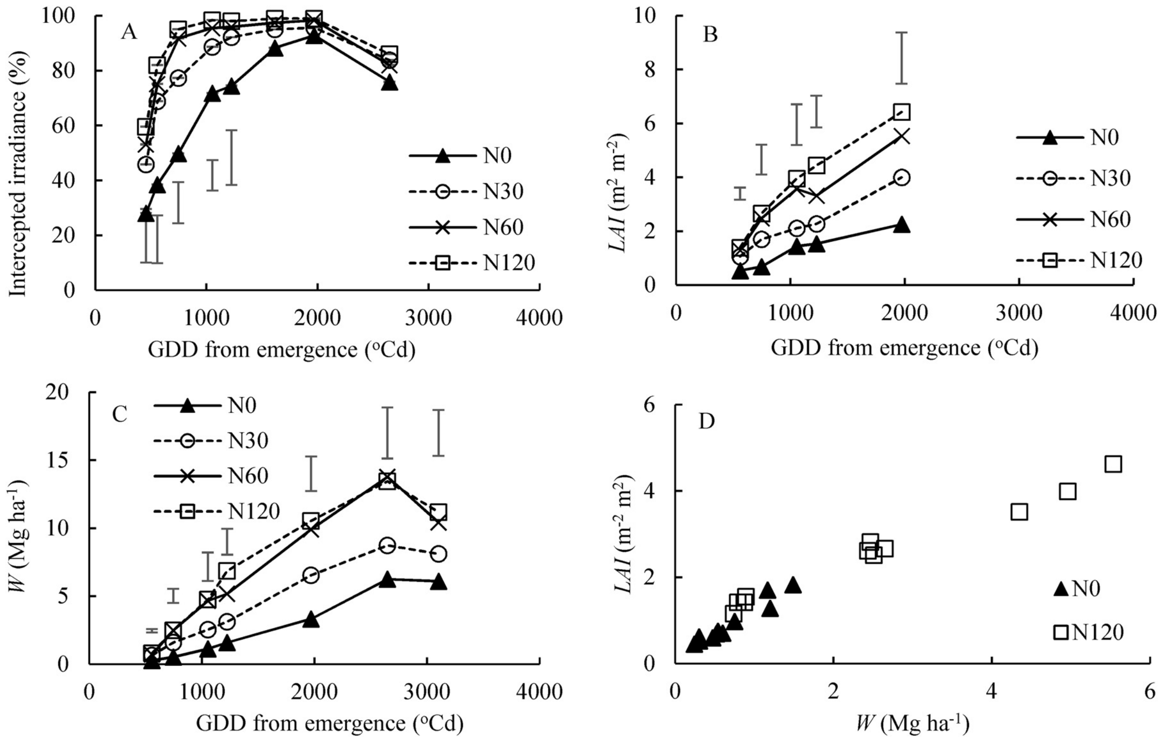

Fig. 4. The effect of nitrogen fertilization on growth. Panels A, B and C indicate the time course of light interception, leaf area index (*LAI*) and biomass yield (*W*), respectively. Panel D is the plot of *LAI* against *W* before flowering. Data presented was collected in the field in Piacenza, Italy in 2015. N0, N30, N60 and N240 stand for nitrogen fertilization rate 0 kg N ha^{-1}, 30 kg N ha^{-1}, 60 kg N ha^{-1} and 120 kg N ha^{-1}, respectively. Vertical bars in Panels A, B and C indicate the Bonferroni LSD for which the effect of nitrogen fertilization was significant at $P < 0.05$.

behaviour that hemp generally shows for above ground and below ground development (Amaducci et al., 2008b). Until canopy closure, *LAI* increases fast at high planting density which goes hand-in-hand with a high *W* accumulation rate and is accompanied by a density independent ratio *LAI*: *W* (Fig. 3). Therefore, canopy closure is reached fast at high planting density. Hemp canopies have a light extinction coefficient (*k*) close to 1 (Amaducci and Stutterheim, 1999; De Meijer et al., 1995). The initially high *LAI* at high planting density results in

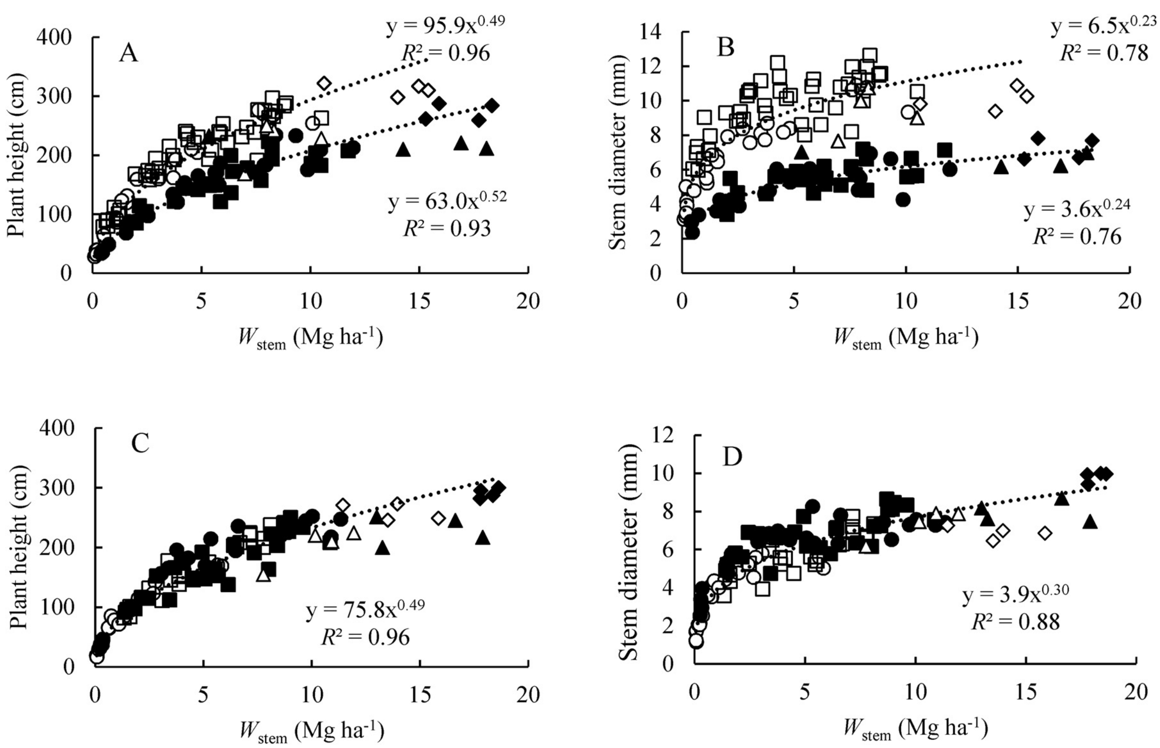

Fig. 5. The relationship between stem yield (W_{stem}) and plant height (A, C), and between (W_{stem}) and stem diameter (B, D). Panels A and B: the open symbols denote planting density at 30 plants m^{-2} while the closed symbols indicate planting density at 240 plants m^{-2}. Panels C and D: the open symbols indicate no fertilization was applied while the closed symbols indicate fertilization rate at 120 kg N ha^{-1}. Data presented was collected in 2015 in Budrio, Italy (■, □), Piacenza, Italy (●, ○), the Czech Republic (◆, ◊) and Latvia (▲, Δ).

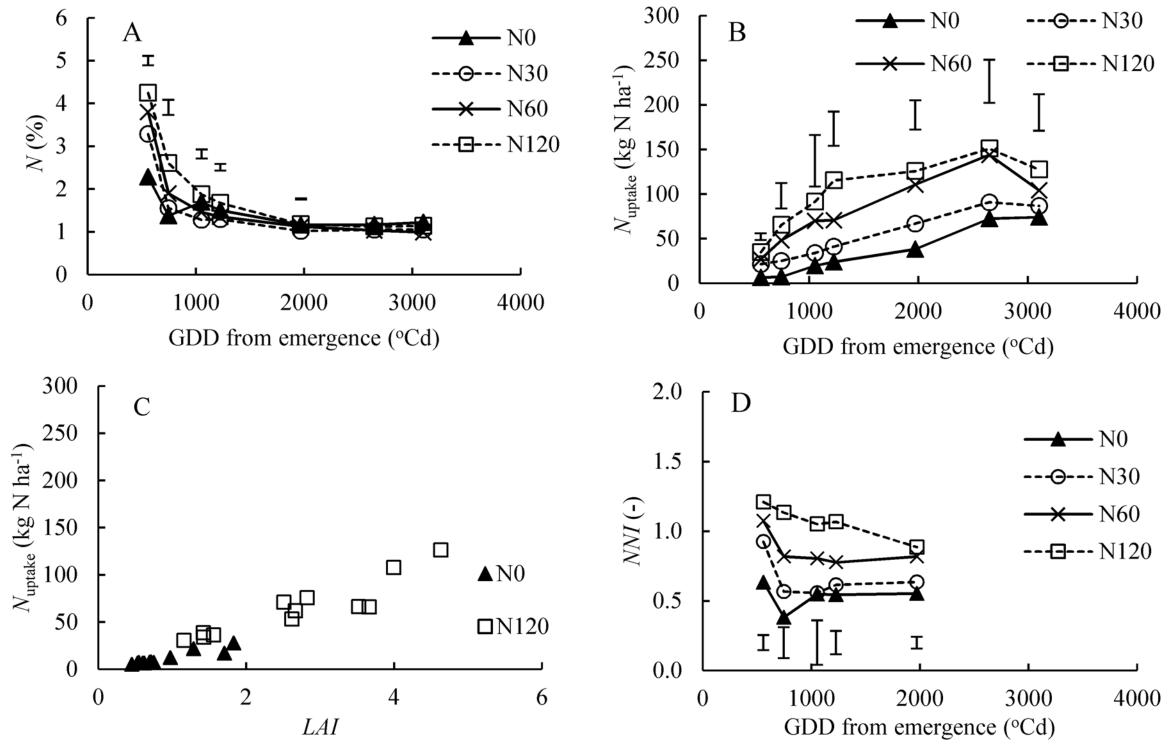

Fig. 6. The effect of nitrogen fertilization on plant nitrogen dynamics. Panels A, B and D indicate the time course of shoot nitrogen concentration (*N*), nitrogen uptake (N_{uptake}) and nitrogen nutrition index (*NNI*), respectively. Panel C is the plot of N_{uptake} against *LAI* before flowering. Data presented was collected in the field in Piacenza, Italy in 2015. N0, N30, N60 and N240 stand for nitrogen fertilization rate 0 kg N ha^{-1}, 30 kg N ha^{-1}, 60 kg N ha^{-1} and 120 kg N ha^{-1}, respectively. Vertical bars in Panels A, B, D indicate the Bonferroni LSD for which the effect of nitrogen fertilization was significant at $P < 0.05$.

severe light competition and reduction in plant number after canopy closure (unshown data and Van der Werf, 1997). As a result, the yield advantage in the first growth phases is lost at high planting density (Fig. 3C). When planting density is extremely low, however, the canopy closure is significantly delayed and *W* is reduced due to the reduction of intercepted radiation during the growing cycle. Consequently, the W_{stem} at 60 plants m^{-2} and 30 plants m^{-2} was the lowest among planting density treatments (Fig. 1A). It should be noted that planting at extremely low density could result in weed competition, which could result in further significant yield reduction (Hall et al., 2014 and authors' experience).

Even when the effect of planting density on W_{stem} is limited, there are very large effects on plant biometrics (Struik et al., 2000). Plants grown at high densities are usually shorter and thinner than those grown at low planting densities (Fig. 5A, B). The results in this study suggest that the effect of planting density on stem diameter is higher than on plant height. For example, when yield was 5 Mg ha^{-1}, at 240 plants m^{-2} stems were 31% shorter and 45% thinner than at 30 plants m^{-2} (Fig. 5A, B). This effect indicates an increase in stem slenderness at high planting density (De Meijer et al., 1995; Westerhuis et al., 2009).

The W_{seed} (seed yield) were not significantly affected by planting density between 30 plants m^{-2} and 240 plants m^{-2} (Table 2, Fig. 2A). This result confirms the observation of Legros et al. (2013) that W_{seed} was independent of planting density until a seeding rate of 40 kg ha^{-1} (corresponding to 200 plants m^{-2}). A constant W_{seed} across a wide range of planting densities is a consequence of the increase in seed yield per single plant with decreasing planting density, which is a result of the increase in inflorescence length (data not shown) and number of branches bearing seeds (Desanlis et al., 2013).

The optimal planting density for dual-purpose production should be

Table 3
Nitrogen content and uptake among organs at seed maturity (H2). Data was obtained in 2013–2015 in Piacenza, Italy. In 2013, nitrogen analysis was only conducted for the samples collected in the plots with planting density 240 plants m^{-2}. Analysis of variance was conducted with nitrogen × year as independent factor.

	2013			2014				2015			
	N0[a]	N50	N100	N0	N30	N60	N120	N0	N30	N60	N120
Nitrogen content (%)											
Stem	0.5 a[b]	0.6 a	0.7 a	0.5 a	0.5 a	0.5 a	0.5 a	0.6 a	0.5 a	0.5 a	0.6 a
Leaf	1.8 ab	2.3 ab	2.3 ab	1.6 ab	1.5 b	1.6 ab	2.4 ab	2.0 ab	1.9 ab	2.0 ab	2.6 a
Seed	3.7 ab	3.7 ab	4.0 a	2.7 c	2.8 c	2.9 bc	3.0 bc	3.3 abc	3.1 abc	2.9 bc	3.4 abc
Nitrogen uptake (kg ha^{-1})											
Stem	42.9 abcd	67.6 ab	74.1 a	7.3 e	17.5 de	32.1 cde	49.8 abc	22.2 cde	28.6 cde	40.1 bcd	50.9 abc
Leaf	42.6 abcd	60.1 a	54.9 ab	9.2 e	16.2 de	24.2 cde	40.4 abcd	27.6 bcde	29.1 bcde	35.8 abcde	49.1 abc
Seed	65.0 a	73.6 a	79.2 a	6.4 b	12.0 b	26.9 b	27.1 b	24.4 b	29 b	28.3 b	27.8 b
Total	150.5 bc	201.3 ab	208.2 a	23.0 f	45.6 ef	83.2 de	117.2 cd	74.2 def	86.7 de	104.3 cd	127.7 cd
Nitrogen utilization efficiency (kg DM kg^{-1} N)											
	86.9 a	75.8 a	76.0 a	98.5 a	108.8 a	105.0 a	107.8 a	82.7 a	97.6 a	104.0 a	89.3 a

[a] N0, N30, N60 and N120 stand for nitrogen application rate 0 kg N ha^{-1}, 30 kg N ha^{-1}, 60 kg N ha^{-1} and 120 kg N ha^{-1}, respectively.
[b] Numbers followed by the same letter in the same row were not significantly different at $P < 0.05$ (Bonferroni test).

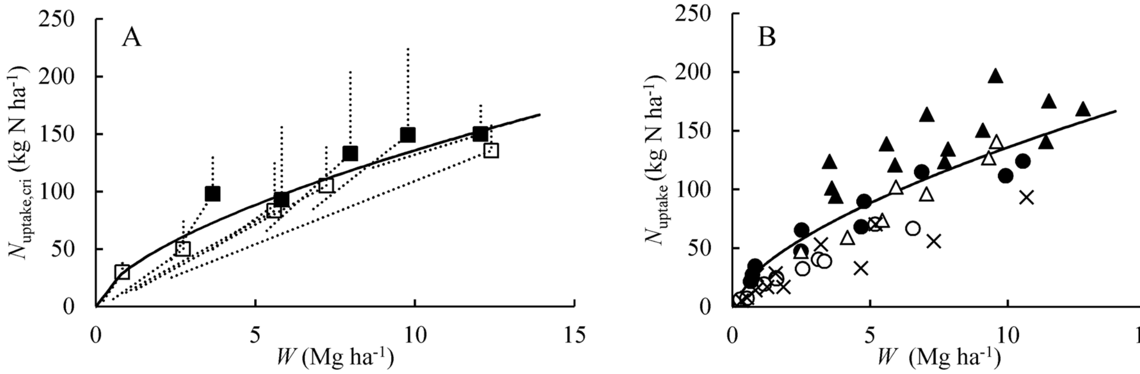

Fig. 7. Panel A: Determination of critial nitrogen uptake ($N_{uptake,cri}$) curve. Dotted lines represent broken stick model for relationship between nitrogen uptake (N_{uptake}) and above ground biomass yield (W); Squares denote $N_{uptake,cri} - W$ points obtained in Budrio (■) and Piacenza (□), in Italy in 2015. The solid line represents the $N_{uptake,cri}$ as a function of W (see Eq. 2). Panel B: The $N_{uptake,cri}$ curve in relation to field measurements of N_{uptake}. The solid line represents the $N_{uptake,cri}$ curve. × denotes data collected in Piacenza-IT, Italy in 2014. Δ and ○ denote nitrogen limiting treatment in Budrio and Piacenza, Italy, respectively, in 2015. ▲ and ● denote nitrogen non-limiting treatment in Budrio and Piacenza, Italy, respectively, in 2015. Note the data collected in 2015 were used to estimate the critial N_{uptake} curve.

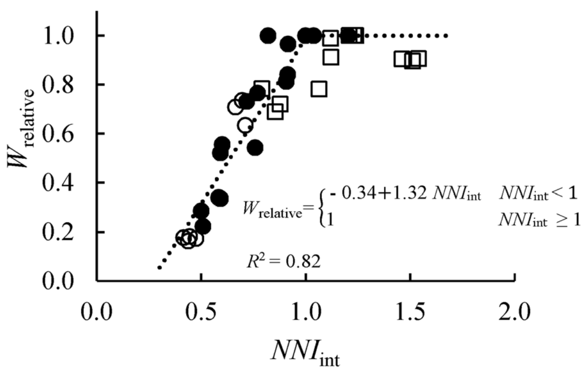

Fig. 8. The relationship between integrated nitrogen nutrition index (NNI_{int}) and relative biomass yield ($W_{relatvie}$; calculated as the ratio of actual and maximum biomass at the same sampling date) at H1 (full flowering). ○ and ● denote samplings in Piacenza, Italy in 2014 and 2015, respectively; □ denotes samplings in Budrio, Italy in 2015.

chosen to optimize both stem and seed yields and also considering that planting density, affecting stem biometrics, interacts with long bast fibre production (Amaducci et al., 2002b; Westerhuis et al., 2009) and with mechanisation of harvest and post-harvest processing (Amaducci et al., 2008a; Amaducci and Gusovius, 2010). It has been recommended to sow at a lower density for hemp seed production (30–75 plants m^{-2}) than for fibre production (90–200 plants m^{-2}) (Amaducci et al., 2015 and references therein). Seed yield in the present study was not significantly affected by plant population across a wide range of plant densities (Tables 2; Fig. 2A); it is therefore recommended to aim at plant populations exceeding 90 plants m^{-2} as lower densities reduce stem fineness, increase the cost of weeding and render mechanical harvesting more difficult due to increased plant height, stem diameter and spike length. On the other hand, although the stems are more slender at higher planting density, densities above 150 plants m^{-2} are not recommended because they do not only increase seed input but also the risk of lodging due to very fine stems (Legros et al., 2013), particularly when soil fertility and/or nitrogen fertilization are high. Considering that stems are more slender at high density and W_{stem} reaches plateaus at a planting density above 120 plants m^{-2} in all environments in the present study (Tables 2; Fig. 1A), the optimum planting density for dual-purpose hemp cultivation could be set at 90–150 plants m^{-2}. It should be noted that the optimal planting density should also consider the effects on fibre quality in terms of post-harvesting processing and final products, which requires further researches.

4.2. The effect of nitrogen fertilization on stem and seed yields

The effect of nitrogen fertilization on W_{stem} interacted with the environment (Fig. 1B), confirming the wide range of responses found in literature (Finnan and Burke, 2013; Prade et al., 2011; Amaducci et al., 2002b; Struik et al., 2000). The results of this study suggest that the effect of nitrogen fertilization on hemp W_{stem} is a consequence of the duration and the intensity of nitrogen deficiency (Fig. 8). Crops respond to nitrogen deficiency through a reduction in resource capture and/or resource use efficiency (Lemaire et al., 2008b). In this study, the ratio LAI: W, which is an approximate measure of radiation capture efficiency, was not affected by nitrogen fertilization, while the N_{uptake}: LAI ratio, which is an approximate measure of radiation use efficiency, decreased when nitrogen was deficient (Figs. 4 D; 6 C). The response of hemp to nitrogen deficiency is similar to that reported in maize and in tall fescue (*Festuca arundinacea*) while it is different to that of wheat (*Triticum aestivum* L.) and oilseed rape, which respond to nitrogen deficiency keeping N_{uptake}: LAI constant while decreasing LAI: W (Lemaire et al., 2008b). The reduction of N_{uptake}: LAI in hemp under nitrogen deficient condition results in a low biomass accumulation rate (Fig. 4C). Consequently, canopy development is restricted and canopy closure is delayed (Fig. 4B). After canopy closure, self-shading occurs and as consequence nitrogen use efficiency of fertilized plots was reduced. Thus, the differences in biomass accumulation rate among nitrogen treatments decreased after canopy closure despite the level of nitrogen deficiency was still high (Fig. 6D).

It has been commonly observed that additional nitrogen fertilization increases hemp plant height and stem diameter (Finnan and Burke, 2013; Amaducci et al., 2008a; Forrest and Young, 2006). The results of this study suggest that plant height and stem diameter are strongly correlated with W_{stem} (Fig. 5C, D). Given that W_{stem} is generally modelled in process based models (e.g. GECROS: Yin and van Laar, 2005), the relationships presented in Fig. 5 are useful to model plant biometrics under different nitrogen regimes.

The positive effect of nitrogen fertilization on W_{seed} (Table 2; Fig. 2B) confirms the results of previous experiments (Aubin et al., 2015; Marija et al., 2011; Vera et al., 2010; Vera et al., 2004). W_{seed} is the product of seed number and seed mass (i.e., 1000 seed weight). It has been reported that nitrogen availability has little effect on hemp seed mass (Marija et al., 2011; Vera et al., 2004). Therefore, we hypothesise that the increase of W_{seed} achieved with additional nitrogen fertilization is a consequence of the positive effect of nitrogen on seed number, as reported for crops such as oilseed rape (Allen and Morgan, 1972; Asare and Scarisbrick, 1995). The reason for the lack of significant effect of nitrogen fertilization on W_{seed} in our study is not clear (Table 2). In 2015 in Piacenza-IT and Budrio-IT, this is probably a consequence of unfavourable weather during the seed filling period. From the beginning of August to September, the crops suffered from limited and unevenly distributed rainfall and high temperature (Supplementary Fig. 1). Drought during the seed filling period results in a reduction in seed dry matter accumulation rate, seed mass and W_{seed} (Plaut et al., 2004). Indeed, in Piacenza-IT in 2015, the seed mass at seed maturity was 37% lower than that measured for the seed used for sowing and the average W_{seed} was 43% lower than that obtained in 2013. Further study is needed to investigate the effect of the interaction between nitrogen fertilization and drought on hemp W_{seed}, which has been reported for crops such as wheat (Ercoli et al., 2008). Ercoli et al. (2008) reported that wheat grain yield reduction by severe post-anthesis water stress was high when combined with additional nitrogen application and was associated with a decrease in kernel weight.

It should be pointed out that determination of W_{seed} at plot level in hemp is very challenging due to the large heterogeneity in the crop (Van der Werf et al., 1995b) and bird predation. Bird predation can only partially be prevented at high cost using nets or bird scarers. Heterogeneity is determined by the contemporary presence of plants of different height, with short plants having short inflorescences with few seeds, and it is aggravated by the duration of hemp seed ripening (from the first seed ripening to the last) which can last for weeks depending on genotype and environmental conditions (Amaducci et al., 2008c) and can cause significant reduction of seed yield by seed shattering or bird predation. The large degree of heterogeneity in our experiments is demonstrated by the high coefficients of variation for W_{seed} ranging from 19% to 36%. These high values might also partly explain the lack of nitrogen fertilization effect on W_{seed}.

While W_{stem} and W_{seed} are restricted by nitrogen deficiency, excess nitrogen supply is not desirable for hemp production. When nitrogen fertilization is excess, stems stay green for longer and this can lead to difficulties in harvesting, longer drying times and difficulties with fibre processing (Legros et al., 2013). Moreover, not only does it increase production cost, excess nitrogen supply has also been widely criticized for its negative environmental effects such as eutrophication of surface water (London, 2005) and gaseous emissions of oxides and ammonia into the atmosphere (Stulen et al., 1998). Therefore, sustainable hemp production requires a supply of nitrogen considering the critical demand.

4.3. Nitrogen demand of hemp

To illustrate hemp nitrogen requirement, a comparison of $N_{critical}$ (critical nitrogen concentration in W) dilution curves between hemp and other crops is presented in Fig. 9A and Supplementary Table 4. In hemp the relationship between W and $N_{critical}$ became exponential at $W > 0.78$ Mg ha^{-1}, a value lower than that found for wheat (Justes et al., 1994) and rice (ssp. japonica) (Ata-Ul-Karim et al., 2013) but close to that of oilseed rape (Colnenne et al., 1998) and sunflower (*Helianthus annuus* L.; Debaeke et al., 2012). Generally the decrease of $N_{critical}$ at increasing W is a consequence of self-shading and of the decreasing ratio LAI: W (Lemaire et al., 2008a). Considering that the LAI: W ratio in hemp remained relatively stable until a high W (> 5 Mg ha^{-1}; Fig. 4D) was reached, the low threshold W (0.8 Mg ha^{-1}) is a sign that self-shading occurred before canopy closure (the incident radiation reached 90% at $W \approx 2$ Mg ha^{-1}) (Fig. 4A, C). This is probably a consequence of the horizontal leaves and the high planting density that has triggered intra-row light competition shortly after emergence.

The position of hemp $N_{critical}$ dilution curve is at the low range of C_3 crops (Fig. 9A and Supplementary Table 4): consistently lower than that of rice (ssp. japonica; Ata-Ul-Karim et al., 2013) and cotton (Xiaoping et al., 2007) and similar to that of the C4 crop maize (Plénet and Lemaire, 1999). The $N_{critical}$ is comparable for hemp and linseed (Flénet et al., 2006) when W is higher than 5 Mg ha^{-1} whereas it is lower for hemp than linseed when W is lower than 5 Mg ha^{-1}. The low position of the hemp $N_{critical}$ dilution curve indicates a relatively high nitrogen use efficiency (Table 3). For example, under non-limiting nitrogen conditions, the minimum nitrogen requirement to produce 10 Mg ha^{-1} of W for hemp is 3%, 7%, 15%, 47% and 58% less than that for linseed, maize, sorghum, oilseed rape and cotton, respectively. The high nitrogen use efficiency of hemp confirms the widely reported low fertilization requirement of this crop (Finnan and Burke, 2013; Prade et al., 2011; Struik et al., 2000).

Although the mechanisms underlying high nitrogen use efficiency for hemp are not clear, we speculate that this is probably the consequence of two reasons. First, hemp contains low structural nitrogen, which is confirmed by the low minimum nitrogen content in W (N_{min}) that is consistently lower than that of cotton (Xiaoping et al., 2007) and rice (ssp. japonica; Ata-Ul-Karim et al., 2013) (Fig. 9B). The low N_{min} of hemp could be explained by the high proportion of stem, on above ground biomass, having very low nitrogen content. Second, hemp has high leaf photosynthetic nitrogen use efficiency at low nitrogen level. In a recent study, Tang et al. (2017) found that the light saturated net leaf photosynthesis rate of hemp was higher than that of cotton when leaf nitrogen content is lower than 2 g N m^{-2}.

4.4. Determination of nitrogen nutrition status

Direct determination of NNI (nitrogen nutrition index) requires very time consuming procedures and these procedures are beyond the expertise and labour availability of farmers (Lemaire et al., 2008a). Thus, a practical estimation is desirable for optimizing hemp fertilization. Several indirect methods to estimate NNI have been summarized by Lemaire et al. (2008a). Since estimation of N_{leaf} (leaf nitrogen concentration) is relatively easy, it has been proposed to correlate it with NNI directly (Ziadi et al., 2009). However, such a direct estimating does not seem correct for hemp because N_{leaf} and N_{stem} (stem nitrogen

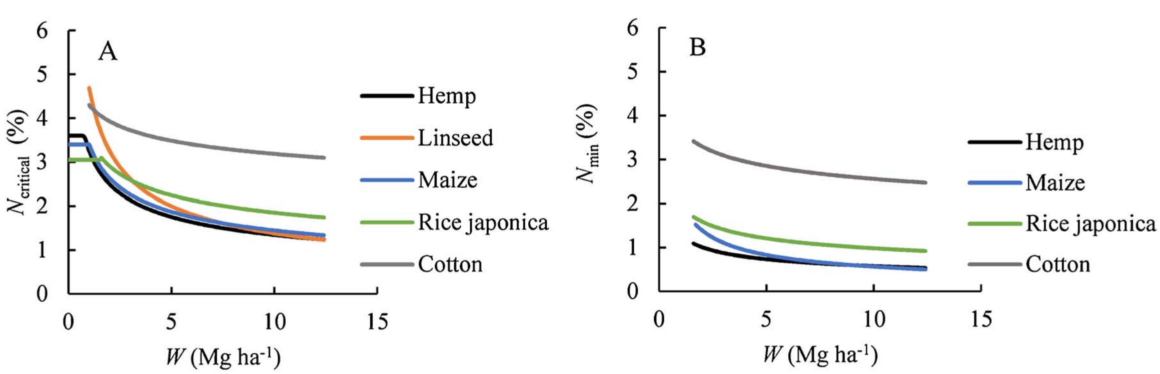

Fig. 9. Illustration of hemp critical nitrogen ($N_{critical}$) dilution curve (Panel A) and minimum nitrogen (N_{min}) dilution curve (Panel B) in comparison with linseed (only in Panel A), maize, rice (ssp. japonica) and cotton. W denotes above ground biomass yield. See Supplementary Table 4 for parameters and references of $N_{critical}$ dilution curve. The N_{min} dilution curve presented for hemp is determined using data collected from the unfertilised treatment.

concentration) are not linearly correlated (Supplementary Fig. 2A). As the relationship between N_{leaf} and N_{stem} is consistent, though, N_{stem} can be approximated using N_{leaf}. Having N_{leaf} and W and an estimate of N_{stem} the *NNI* can in turn be estimated. This empirical method resulted in a better estimation of *NNI* than by the correlation between N_{leaf} and *NNI* (Supplementary Fig. 3).

In agronomic practice, a fast and indirect estimation of N_{leaf} is required. *SPAD*, an indicator of the leaf chlorophyll concentration, could be a useful index for quantifying N_{leaf} as a good correlation between N_{leaf} and *SPAD* was observed (Supplementary Fig. 2B). However, despite the fact that many authors found a good correlation between *SPAD* and N_{leaf} (Lin et al., 2010; Matsunaka et al., 1997), one should be aware that this relationship is highly variable from one study to another due to differences in environmental conditions and genotypes. In an attempt to eliminate the effect of environmental conditions and genotypes on the diagnosis of plant nitrogen status through *SPAD*, several *SPAD* based indexes have been proposed such as: normalized *SPAD* index (Yuan et al., 2016) and positional differences chlorophyll measurements index (Zhao et al., 2016). Further study is necessary to evaluate the performance of these *SPAD* based indexes on hemp nitrogen status diagnosis before a nitrogen nutrition status based decision on nitrogen management can be attempted.

5. Conclusions

The effects of planting density and nitrogen fertilization on hemp stem and seed productions were assessed in eight environments (combinations of year and location) at five contrasting locations throughout Europe. The effects of these two factors on hemp stem and seed yields neither interacted with each other nor with cultivar. Changing planting density over a wide range had limited effect on both stem and seed yields while plant height and stem diameter decreased with increasing population. The optimum planting density for dual-purpose hemp cultivation could be set at 90–150 plants m^{-2}. Nitrogen deficiency reduced stem yield and seed yield. The effect of nitrogen deficiency on plant height and stem diameter was in accordance with its effect on stem yield. Hemp has a high nitrogen use efficiency and 60 kg N ha^{-1} was generally sufficient in the tested environments for dual-purpose hemp cultivation. However, optimization of nitrogen fertilization requires assessment of plant nitrogen status. Direct determination of nitrogen status for hemp are too complex while *SPAD* based diagnosis techniques requires further investigations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2017.06.033.

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