Agronomical evaluation and chemical characterization of *Linum usitatissimum* L. as oilseed crop for bio-based products in two environments of Central and Northern Italy

Silvia Tavarini,[1] Luciana G. Angelini,[1] Nerio Casadei,[2] Paolo Spugnoli,[3] Luca Lazzeri[2]

[1]Department of Agricultural, Food and Environmental Sciences, University of Pisa; [2]Research Centre for Industrial Crops, Council for Agricultural Research and Agricultural Economy Analysis, Bologna; [3]Department of Agricultural, Food and Environmental Systems Management, University of Firenze, Italy

Abstract

In the recent years, new perspectives for linseed (*Linum usitatissimum* L.) are open as renewable raw material for bio-based products (Bb), due to its oil composition, and the interesting amounts of co-products (lignocellulosic biomass). Therefore, the possibility to introduce linseed crop in two environments of central and northern Italy, traditionally devoted to cereal cultivation, has been evaluated. Two-years field trials were carried out in the coastal plain of Pisa (Tuscany region) and in the Po valley (Bologna, Emilia Romagna region), comparing two linseed varieties (Sideral and Buenos Aires). Agronomical evaluation (yield and yield components), seed and oil characterization (oil, protein content, and fatty acid composition), together with carbon (C) and nitrogen (N) content of the residual lignocellulosic biomass were investigated. The two varieties, grown as autumn crop, showed a different percentage of plant survival at the end of winter, with Sideral most resistant to cold. The achieved results showed significant influence of cultivar, location and growing season on yield and yield components, as well as on chemical biomass composition. In particular, Sideral appeared to be the most suitable variety for tested environments, since higher seed yield (3.05 t ha^{-1} as mean value over years and locations) and above-ground biomass (6.98 t ha^{-1} as mean value over years and locations) were recorded in comparison with those detected for Buenos Aires (1.93 and 4.48 t ha^{-1} of seed production and lignocellulosic biomass, respectively). Interestingly, in the northern area, during the 1st year, Buenos Aires was the most productive, despite its low plant survival at the end of winter, which determined a strong reduction in plant density and size. In such conditions, the plants produced a larger number of capsules and, consequently, high seed yield (3.18 t ha^{-1}). Relevant differences were also observed between the two years, due to the variability of climatic characteristics (temperature levels, and moisture regimes). All these findings confirmed as, in linseed, yield and yield components are quantitatively inherited and influenced by both genotype and environment (location and climate). Varietal and environmental effects were also recorded for oil content and yield, and, generally, good oil percentages, for both genotypes, were found (ranging from 44 to 49% on dry matter basis). Oil from the two varieties was characterized by a stable proportion of polyunsaturated fatty acids with a high content of alpha-linolenic acid (more than 57%), that makes this oil suitable to be used in paints, resins, varnishes, linoleum, polymers and oleochemicals. Finally, our results pointed out as above- and below-ground biomasses, were different in terms of quantity, and chemical characteristics (N, C and C/N ratio). Interesting amounts of N and C could return into the soil by crop residues (stem portions and roots), thus underling the possibility to maintain and/or increase the soil organic matter pool.

Correspondence: Luciana G. Angelini, Department of Agricultural, Food and Environmental Sciences, University of Pisa, Italy.
Tel.: +39.050.2218901. E-mail: luciana.angelini@unipi.it

Key words: Bio-based products; linseed; yield and yield components; lignocellulosic biomass.

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Introduction

Bio-based products (Bb) are increasingly requested by consumers and industry in substitution of fossil-based raw materials and have triggered the interest of governments worldwide to support the transition to a bio-based economy (Sanders and van der Hoeven, 2008). A bio-based product is defined as a product based on renewable resources (vegetal, animal or fungal) according to the *biomass cascading* approach. The concept of *biomass cascading* contemplates the full use of biomass including the co-products, favouring highest value added and resource efficient products, such as bio-based products and industrial materials (pharmaceuticals, nutraceuticals, fine chemicals, cosmetics, agrochemicals, biomaterials), over bioenergy (Commission

Staff Working Document, 2012; Essel *et al*., 2014). However, not all biomass feedstocks are suitable for producing high added value products (*e.g*., contaminated or very heterogeneous residues), and the energy sector - which is at least one order of magnitude larger than the chemical sector - should be considered (Kamm and Kamm, 2004; Fernando *et al*., 2006; Star AgroEnergy, 2012). This whole and complex way to handle the biomass, increases the resource efficiency and maximizes the environmental benefits (Carus *et al*., 2014). In this scenario, new perspectives for conventional and new non-food oleaginous crops are open as based materials for renewable feedstocks, taking into account their renewability and biodegradability as a fundamental tool for a new green chemistry (Wool and Sun, 2005; Espinosa and Meier, 2011). Plant oils are important bio-resources for utilization in several industrial sectors (surfactants, plasticizers, emulsifiers, detergents, lubricants, adhesives, cosmetics, fine chemicals), giving good yields for both the oil and the residual lignocellulosic biomass (epigeal and hypogeal), with relatively low cultivation inputs and environmental friendly cultivation methods (Zanetti *et al*., 2013). Another interesting feature is the possibility of their cultivation on low fertility agricultural areas and/or marginal lands with satisfactory yields even in rotation with food cereal production.

Among oilseed crops, linseed (*Linum usitatissimum* L.), one of the most versatile and useful crop (Genser and Morris, 2003), has gained considerable attention as a potential oilseed feedstock for advanced bioproducts, since diverse materials (*i.e*., biolubricants, bioplastics, biochemicals, biomaterials and biofuels as well) for several potential applications can been produced (Zanetti *et al*., 2013). Linseed is an annual winter or spring crop, originating from Middle East (Iran or Kurdistan), cultivated for its seed, and, as a dual-purpose crop, also for its fibrous stems. Basically, linseed products might be used in different branches of industry, and its utilization for food, feed, and fiber, has been recently deeply reviewed (Chung *et al*., 2005; Jansman *et al*., 2007; Carter *et al*., 2008; Singh *et al*., 2011). Linseed is usually harvested when the seed is sufficiently dry, and the residual straw, which represents a considerable part of the biomass in the field, can be valorised to obtain short fibres used in non-woven applications (specialist papers, composite materials, and biodegradable products), thus increasing farming income and the system sustainability (Sankari, 2000a, 2000b; Zuk *et al*., 2015). Finally, the seed meal, derived from screw-pressed oil extraction, is characterised by a good protein content (about 36% with 85% digestibility), residual oil (from 7 to 10%), and other minor molecules, such as phenolic acids and flavonoids, that could find increasing application in the feed, food, cosmetic, and pharmaceutical industries (Singh *et al*., 2011).

Although a significant increase of linseed production was noticed in Eastern Europe (more than 2.5 fold during last five years), such as Ukraine, Belarus, and Russian Federation as well (Zuk *et al*., 2015), it is a relatively new crop for many countries of the Mediterranean Europe, Italy included. While fiber flax cultivars are preferably grown in cool and moist climate conditions, linseed cultivars can be grown in many environments due to their plasticity, mainly in temperate climate regions, where they can explicate the best agronomic performances in terms of seed yield, yield components, oil content and composition (D'Antuono and Rossini, 1995; Casa *et al*., 1999; Adugna and Labuschagne, 2003; Cross *et al*., 2003). To date, winter-hardy varieties of linseed, and also yellow-seeded cultivars with altered fatty-acid profiles, are being introduced to commercial cultivation; the latter are intended for edible oil production, but industrial uses are also likely (Ali *et al*., 2011). Even if linseed shows a great phenotypic plasticity in terms of growth and yield components, in response to different agrotechniques (Diepenbrock and Pörksen, 1992), the choice of the suitable variety to particular environmental conditions is extremely important for a successful linseed cultivation, since the yield response of each genotype is strongly related to the variations of pedo-climatic conditions of the cultivation site (soil fertility, water availability, temperature, photoperiod, light intensity, crop management, *etc*.) (Crossa *et al*., 1991; Berti *et al*., 2010).

Therefore, the aim of this study was to evaluate the agronomical and qualitative responses of two linseed varieties, grown in two environments of central (coastal plain of Pisa province, Tuscany region) and northern (Po valley, Bologna province, Emilia Romagna region) Italy, traditionally devoted to cereal cultivation. Through 2-years large field experiments, agronomical evaluation (biometric characteristics, yield and yield components), seed and oil characterization (oil and protein content, and fatty acid composition), together with carbon (C) and nitrogen (N) content of the residual lignocellulosic biomass were investigated.

Materials and methods

Field experiments and plant materials

Two varieties of linseed (Sideral and Buenos Aires) were studied under field conditions in two environments, representative of the pedo-climatic characteristics of central and northern Italy, during 2012-2013 and 2013-2014 growing seasons, as autumn crop under rotation with wheat.

The two linseed varieties were chosen on the basis of their adaptability to the environmental conditions of the tested areas, through previous screenings. The var. Sideral (registered in EC Common catalogue of varieties and evaluated in France), is commercially available (Semfor s.r.l., Verona, Italy), characterised by highly resistance to cold and lodging, high rusticity, and very early ripening with blue-violet flowers and brown coloured seeds. The var. Buenos Aires (selected by and belonging to the germplasm collection of CREA-CIN, Bologna, Italy) shows a lower cold resistance than Sideral, and it is characterised by a very early ripening, with white flowers and yellow coloured seeds.

The cultivation site of central Italy was located at San Piero a Grado, in the Pisa coastal plain (Tuscany region, 43°40'N latitude; 10°19'E longitude, altitude 1 m a.s.l. and 0% slope) at the *Enrico Avanzi* Centre for Agro-Environmental Research (CiRAA) of the University of Pisa. The area was characterized by flat land with alluvial deep silt-loam soil. The soil was a typic Xerofluvent, according to the unified soil classificaiton system of the United States Department of Agriculture (USDA) (Soil Survey Staff, 1975), representative of the lower Arno river plain, characterized by a superficial water table 120 cm deep in the driest conditions. The northern site was located at Budrio (Bologna, Emilia Romagna region, Italy) in the Po valley (44° 32' 13'' N, 11° 29' 40'' E, altitude 29 m. a.s.l. and <1% slope) at the Experimental Farming of the CREA-CIN at Budrio (Bologna). The area was characterised by flat morphology, with alluvial loamy soil, representative of the fertile Po valley. The physical and chemical soil characteristics were examined at the beginning of the experiment and soil samples were collected in each plot at 30 cm depth. Total nitrogen was evaluated by the macro-Kjeldahl digestion procedure (Bremner and Mulvaney, 1982). Soil organic carbon (SOC) was determined using the modified Walkley-Black wet combustion method (Nelson and Sommers, 1982). Soil organic matter was estimated by multiplying the SOC concentration by 1.724 (Nelson and Sommers, 1982). Changes in minimum, maximum and mean air temperatures and total rainfall were recorded throughout the field experiments by a weather station located nearby each experimental site.

In each environment, the crops were subsequent to wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.], assuming a rotation with cereals. The seed amount for sowing was calculated by taking into account the measured germination and thousand seed weight. In the 1st year of cultivation, sowing was accomplished on 8th and 5th October 2012, in Pisa and Bologna, respectively. Similarly, in the 2nd growing season, the sowing was realized on 31th and 16th October 2013, in central and northern environment, respectively. The cropping techniques and mechanisation methods were defined in relation to the specific characteristics of the area, with the aim of performing the experiments under low input management. Fertiliser rates were calculated according to soil test levels. The agro-techniques adopted are given in Table 1. The experiment was laid out as open field trial with a single plot design, being the total field area divided in two main plots (the size of each plot was 1000 m^2) corresponding to each cultivar, even if the trials and the results were evaluated also by a four-replicate randomised samplings. At seed maturity, when average seed moisture was lower than 9%, four sampling areas of 2 m^2 per plot were manually harvested, excluding outer-rows, to assess harvestable crop yield. In Pisa, the harvesting time was accomplished on 3rd July 2013 and 25th June 2014, while in Bologna on 17th July 2013 (for both varieties) and 12th and 24th June 2014, for Buenos Aires and Sideral, respectively. Plant density, plant height and productive characteristics (above- and below-ground biomass, seed yield, yield components and harvest index) were evaluated. Fresh weight was measured, and plants subsequently allowed to dry into a ventilated oven (40°C) for dry weight determination and evaluated for their moisture content. To evaluate potential seed yield, the plants were threshed by a fixed machine, using sieves suitable for small seeds. Apparent harvest index (HI) was calculated as:

(seed weight/mature plant weight) × 100.

Thousand seed weight was assessed according to the international rules for seed testing (ISTA, 2005). After sampling, the entire surface was harvested using a reap thresher plot-machine and the yield, referred to one hectare, is reported as actual yield.

Fatty acid composition and oil content determination

The oil was extracted from ground seed by hexane and trans-methylated in 2N KOH methanol solution (Conte *et al*., 1989). Fatty acid methyl esters were evaluated by a gas chromatography-FID detector (Carlo Erba HRGC 5300 MEGA SERIES) on a capillary column Restek RT x 2330 (30 m × 0.25 mm × 0.2 μm) with oven temperature programming (170°C initial temperature for 12 min, followed by a gradient of 20°C/min to 240°C, for 3 min), helium as carrier gas at 1 mL/min and split mode 40:1. The detector and injector temperature was 260°C.

Standards were used for identification of individual fatty acids. The internal normalization method (ISO 5508, 1998) was used to determine the fatty acid composition. The oil content was determined by the standard Soxhlet extraction method using n-hexane as solvent.

Determination of total carbon, nitrogen and protein content

Grounded samples were loaded (0.15 grams) into tin foil cups and analysed by LECO Truspec® CHN Analyzer. All setting parameters followed the *Organic Application Note* (LECO). Data analysis was performed through NetOp® software and results expressed as percentage on dry weight (g) (http://www.leco.com/index.php/support/library). The protein content was expressed as percentage on dry matter and calculated from nitrogen using the conventional factor of 6.25 (Sosulski and Imafidon, 1990).

Statistical analysis

All the variables were subjected to the analysis of variance (ANOVA) using the statistical software CO-STAT Cohort, 2002 (CoHort Software, Monterey, CA, USA). A factorial design with cultivar (C), location (L) and growing season (G) as main treatments was used. The effects of C, L, G and their reciprocal interactions were analysed by three-way completely randomised ANOVA. Means were separated on the basis of least significance difference test only when the ANOVA F-test per treatment was significant at the 0.05 probability level (Gomez and Gomez, 1984). Linear regression analyses using GraphPad PRISM Version 4.0 (GraphPad Software, Inc., La Jolla, CA, USA) were performed in order to evaluate the relationships between the oil and crude protein content.

Results and discussion

Pedo-climatic conditions of cultivation sites

The soil physical and chemical characteristics of the two sites are reported in Table 2. In Pisa, the soil was characterised by loam texture, with medium level of total nitrogen and an average content of organic matter. In Bologna, the soil was silty clay loam with good organic matter

Table 1. Linseed crop management adopting in the two environments of central and northern Italy.

Field set-up management - Pisa	Feld set-up management - Bologna
Shallow ploughing (30 cm)	Shallow ploughing (25-30 cm)
Disk harrowing	Disk harrowing
Mineral fertilizers before seeding (80 kg ha^{-1} of P_2O_5 and K_2O as triple superphosphate and potassium sulphate, respectively)	Organic fertilizer before seeding (600 kg ha^{-1} equal to 11 kg N ha^{-1})
Tine harrowing	Tine harrowing
Sowing (rate of 40 kg ha^{-1} on 15 cm spaced rows using a plot drill for wheat, in both years of cultivation)	Sowing (rate of 40 and 45 kg ha^{-1} on 15 cm spaced rows using a plot drill for wheat, in the 1st and 2nd year, respectively)
Pre-emergence chemical weeding in the 2nd year (p.a. metazaclor at the rate of 2 L ha^{-1})	No herbicide treatment
Nitrogen fertilization after seeding (80 kg N ha^{-1} split in two applications as ammonium nitrate)	Nitrogen fertilization after seeding (50 kg N ha^{-1} as ammonium nitrate)
No desiccant treatment	Desiccant treatment before harvest only in the first year of cultivation
Mechanical harvest with a plot thresher machine	Mechanical harvest with a plot thresher machine

and total nitrogen contents. The coastal plain of Pisa province is characterised by flat lands and Mediterranean climate, with minimum low temperatures in January (2°C as mean monthly value), and maximum high temperatures in July (29°C as mean monthly value). Rainfalls are mainly concentrated in autumn and spring time (941 mm year^{-1}). During summer (July-half August), a dry period generally occurs, with low rainfall and high air temperatures. The Po valley in Bologna province is a plain area characterised by a continental climate, with low mean temperatures during winter (0-4°C) and minimum temperatures below zero and common flogs. Rainfalls are mainly concentrated in spring and autumn, and during summer a dry period generally occurs with high air temperatures. Monthly meteorological conditions of the two growing seasons (from October to July) were showed in Figure 1. At Pisa, in the 1st year, the mean temperatures were about 13.4°C, while the minimum and maximum values ranged between 8.1 and 18.6°C (Figure 1A). Rainfalls were particularly abundant (about 1326.8 mm) and mainly concentrated in November, January and May. The 2nd growing season was warmer than the previous one with a mean temperature of 14.2°C and minimum and maximum equal to 8.7 and 19.8°C (Figure 1B). Rainfalls, even if abundant, were lower than in the previous year (1179.4 mm) with January, February and July as the wettest months. In Bologna (Figure 1C and D), the 1st year of cultivation was characterised by a mean temperature of 11.6°C and minimum and maximum temperatures of about 6.8 and 16.7°C. Rainfalls were about 680 mm, mainly concentrated at the end of the winter (February and March) (Figure 1C). In the 2nd year, an increase of temperatures was registered (about 13.1, 7.6 and 18.6°C, mean, minimum and maximum temperature respectively) together with more abundant rainfall (877.2 mm), particularly in October, November, January and also in July.

Effect of the main variability factors and their reciprocal interactions

In Table 3, the results from the three-way ANOVA, taking into account cultivar (C), location (L), growing season (G), and their reciprocal interactions (C × L; C × G; L × G; and C × L × G), have been reported. Among the different factors of variation, the cultivar signifi-

Table 2. Physical and chemical characteristics of the soils of the two environments where the experimental trials were carried out.

	Pisa	Bologna
Sand (%)	43.6	7.0
Clay (%)	11.3	33.0
Silt (%)	45.1	60.0
SOM (%)	1.72	2.09
$CaCO_3$ (%)	3.12	10.71
N_{tot} (g kg^{-1})	1.08	1.43
Bulk density (g cm^{-3})	1.48	1.30

SOM, soil organic matter.

Table 3. Results from three-way completely randomised analysis of variance considering the effect of the factor of variation (cultivar, location, growing season and their reciprocal interactions) on the various parameters measured in this study.

Parameters	Cultivar (C)	Location (L)	Growing season (G)	C x L	C x G	L x G	C x L x G	CV %
df	1	1	1	1	1	1	1	
Plant density	***	***	n.s.	***	***	***	***	11.825
Plant height	**	***	**	*	n.s.	**	n.s.	9.002
Plant winter survival	***	***	***	***	n.s.	n.s.	***	7.397
Potential seed yield	***	***	n.s.	***	***	***	ns	9.958
Seed moisture at harvest	*	***	***	***	***	***	***	2.760
Num. of capsules per m^2	***	n.s.	n.s.	n.s.	n.s.	*	***	7.617
Num. of seeds per capsule	***	***	n.s.	***	***	***	**	9.643
1000-seeds weight	***	n.s.	n.s.	***	***	**	*	3.166
Above-ground dry biomass	***	***	***	***	***	***	*	7.388
Below-ground dry biomas	***	n.s.	*	n.s.	n.s.	***	n.s.	12.875
Harvest index	n.s.	**	***	n.s.	***	n.s.	**	50.305
Seed oil content	***	***	n.s.	***	n.s.	***	n.s.	1.089
Oil yield	***	***	n.s.	***	***	***	n.s.	7.686
Crude protein content	*	***	n.s.	*	**	***	**	3.814
Straw C content	***	n.s.	***	***	***	***	n.s.	1.583
Straw N content	***	***	**	***	**	***	***	7.978
Straw C/N	***	*	n.s.	***	***	***	***	9.296
Potential N addition by straw	***	***	***	***	n.s.	**	***	6.922
Potential C addition by straw	***	***	***	***	***	***	**	6.751
Root C content	***	***	***	***	n.s.	***	***	1.000
Root N content	***	***	***	n.s.	***	***	***	3.218
Root C/N	***	***	***	***	***	***	***	3.562
Potential N addition by roots	***	***	*	n.s.	***	***	***	0.568
Potential C addition by roots	***	n.s.	**	**	n.s.	***	n.s.	13.208

CV, coefficient of variability; df, degree of freedom; C, carbon; N, nitrogen. *, **, ***, n.s. = significant at $P \leq 0.05$; ≤ 0.01; ≤ 0.001 and not significant, respectively, according to analysis of variance.

cantly affected all the characteristics, exception given for the harvest index. Similarly, the location played a key role, affecting most of the parameters, except for number of capsules per m^2, 1000-seed weight, below-ground biomass, straw C content, and potential C additions by the roots. Growing season was also an important factor affecting plant height, plant winter survival, seed moisture, above- and below-ground biomasses, harvest index, chemical composition of both above- and below-ground biomass, and root C/N ratio, but not the potential seed yield. The C×L interaction significantly influenced several parameters, except for the number of capsules per m^2, the below-ground biomass, HI, the N content and the potential N addition by roots. Plant density, potential seed yield, number of seeds per capsule, 1000-seed weight, above-ground biomass, HI, oil yield, protein content, and the majority of chemical characteristics were all affected by C×G interaction. Regarding L×G interaction, only plant winter survival and harvest index were not significantly influenced. Finally, the C×L×G interaction significantly influenced most of the parameters considered, but not the potential seed yield.

Yield and yield components

Significant differences between genotypes and environments were observed for the potential seed yield and for the most of the yield components (Tables 3 and 4). In the 1st growing season, both varieties successfully survived after the winter period in Pisa, showing a good resistance to the winter temperatures, which rarely fall below zero in this environment. On the other hand, the very high rainfall registered in January 2014 in this site (355.4 mm, whose 228 mm from 14 to 19 January) and the consequent soil flooding, caused damage and mortality of established plants with a reduction of plant density (Table 4). On the contrary, the more severe minimum winter temperatures registered in Bologna during the 1st year of cultivation, with several days below zero and snowfalls (from December to February), caused serious plant frost damages, that negatively affected Buenos Aires plant survival, with a plant loss of 75% at the end of winter (Table 3). In the 2nd year, the winter minimum temperatures in Bologna were less severe than the previous year (with mean minimum temperatures below zero only in few days of December), and, as a consequence, Buenos Aires showed a better plant survival (62%). Differently to that observed for Buenos Aires, Sideral showed a good plant survival (89% and 80%, in the 1st and 2nd year, respectively), confirming to be more resistant to cold in Bologna.

These data highlighted, as in both environments, Sideral appeared to be the most suitable variety in terms of winter survival, showing a great cold resistance. In both years of cultivation, Sideral confirmed higher seed yield (3.05 t ha^{-1} as mean value over years and locations) and lignocellulosic above-ground biomass production (6.98 t ha^{-1} as

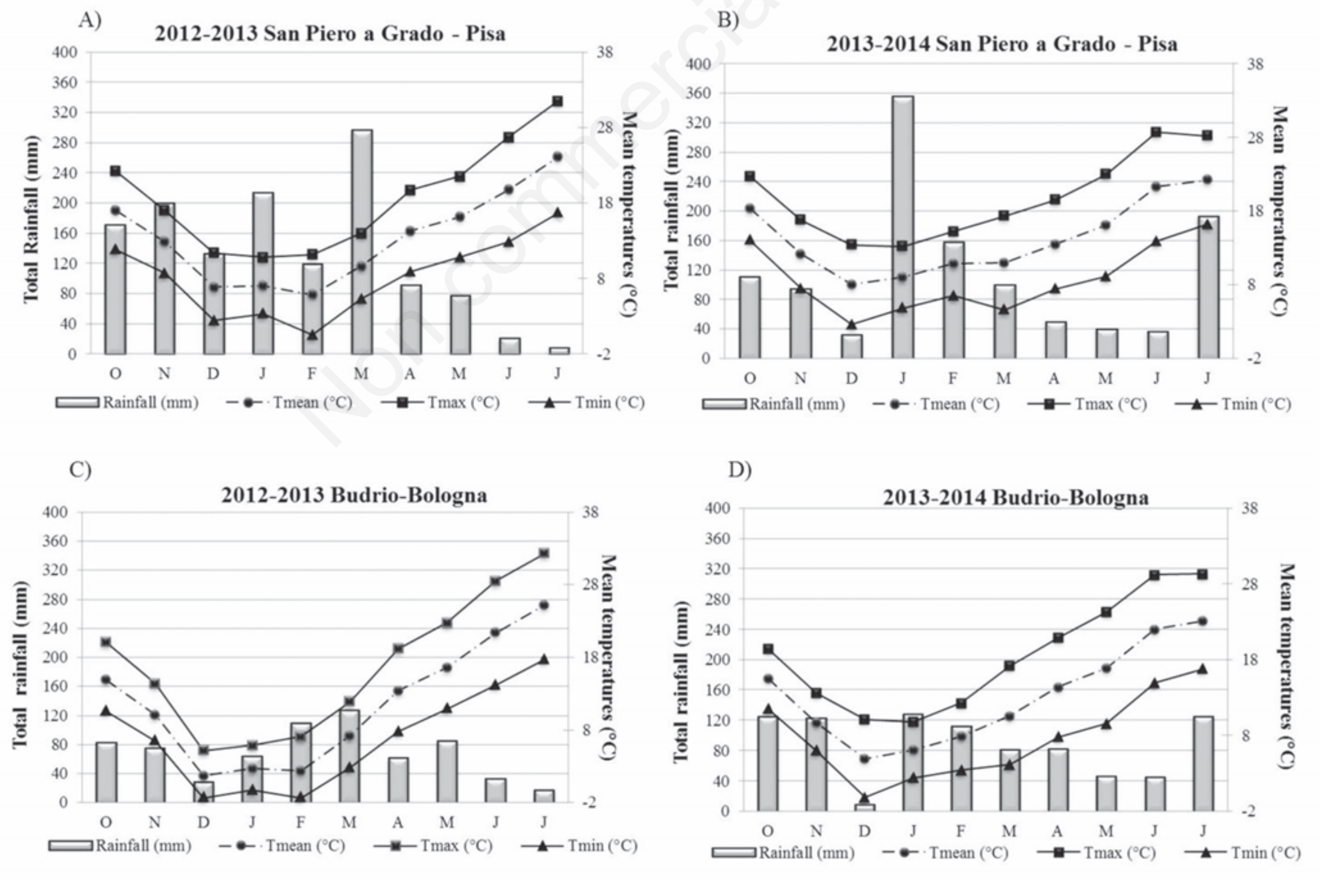

Figure 1. Meteorological data (monthly rainfall and monthly mean temperatures) for 2012-2013 and 2013-2014 growing seasons at Pisa (A and B) and Bologna (C and D).

mean value over the years and locations), than Buenos Aires (1.93 and 4.48 t ha^{1} of seeds and lignocellulosic biomass, respectively). In addition, in Pisa it was possible to observe that, in the 1st growing season, yield of seeds and lignocellulosic residues were lower than in the 2nd year for both genotypes. This could be due to the drought stress which occurred in 2013 during seed filling stage (June-July), when mean maximum temperatures reached 31.5°C and very low total rainfalls (8.2 mm) occurred (Figure 1). It is reported that temperature is the most important factor influencing yield, oil content, maturity period, plant height, fatty acid levels and diseases of linseed (Green, 1986; Adugna, 2000; Adugna and Labuschagne, 2003). Low rainfall and high temperatures during the seed filling period can have a significant and negative effect on seed yield (Casa *et al*., 1999; Dordas, 2010) and can accelerate maturity, reducing seed size and oil content (Luhs and Friedt, 1994). In addition, high temperature at flowering stage was reported to be deleterious for capsule setting, depress seeds per capsule and seed weight (Green, 1986).

Interestingly, in Bologna, in the 1st year of cultivation, Buenos Aires appeared more productive (3.18 t ha^{-1}) than Sideral (2.32 t ha^{-1}), in spite of the low plant survival of the first one at the end of winter, which considerably reduced its plant density (150 plants m^{-2}) (Table 4). In such conditions, the plants showed a reduced size, producing more branches, which stimulated the formation of a larger number of capsules and seeds on the stems.

Considering the C×L interaction, it was possible to observe that, in the 1st year of trial, Buenos Aires cultivated in Pisa appeared the least productive in terms of seed yield and above-ground biomass. In the 2nd season, Buenos Aires grown in Bologna was the least productive in terms of seed yield, while Sideral cultivated in Pisa showed the highest yields, with the highest number of capsule per m^2.

However, it is important to take into account that, in order to obtain high yields, the seed filling period is very important for linseed. In general, a longer grain filling period leads to higher yields and also to better quality. One of the most important disadvantages of growing linseed in a Mediterranean climate is that the flowering period falls in May, when evapotranspiration is high and the crop experiences water stress (Casa *et al*., 1999). Thus, it is important that the plant should flower as early as possible, in order to obtain maximum yield. Anyway, the yields observed in both environments, were consistent with those reported in literature (Lafond *et al*., 2008; Berti *et al*., 2010). Luhs and Friedt (1994) have estimated the yield of modern linseed cultivars can reach up to 3 ton/ha under optimum conditions, though realization of this potential is often limited by input level and ecological conditions. Regarding actual seed yield, obtained by mechanical harvest of the entire field plot, in both years and locations, Sideral was the most productive, reaching its highest performance in the 2nd year of cultivation

Table 4. Yield and yield components (results are the means ± standard deviation of four replicates) in two linseed varieties (Sideral and Buenos Aires) in central (Pisa) and north (Bologna) Italy during two consecutive growing seasons.

	Sideral		Buenos Aires	
	Pisa	Bologna	Pisa	Bologna
2012-2013				
Plant density (plant n. m^{-2})	452.2 ± 63.8^{b}	653.7 ± 70.6^{a}	392.9 ± 44.5^{b}	150.1 ± 27.0^{c}
Plant height (cm)	77.4 ± 2.3^{ab}	82.0 ± 8.4^{a}	72.5 ± 8.4^{b}	60.0 ± 4.2^{c}
Plant winter survival (%)	71.6 ± 9.8^{b}	89.1 ± 4.9^{a}	95.1 ± 2.1^{a}	25.0 ± 3.3^{c}
Potential seed yield (t ha^{-1})	3.06 ± 0.26^{a}	2.32 ± 0.26^{b}	1.27 ± 0.16^{c}	3.18 ± 0.24^{a}
Actual seed yield (t ha^{-1})°	1.39	0.94	0.60	0.75
Seed moisture at harvest (%)	9.41 ± 0.20^{a}	6.01 ± 0.80^{c}	8.20 ± 0.39^{b}	5.10 ± 0.58^{d}
Num. of capsules per m^2	7370.9 ± 651.2^{a}	7447.0 ± 673.0^{a}	4714.8 ± 147.8^{c}	6100.1 ± 251.0^{b}
Num. of seeds per capsule	6.60 ± 0.78^{a}	5.10 ± 0.48^{b}	3.69 ± 0.77^{c}	6.35 ± 0.32^{a}
1000-seeds weight (g)	6.29 ± 0.51^{c}	6.10 ± 0.40^{c}	7.29 ± 0.14^{b}	8.20 ± 0.11^{a}
Above-ground dry biomass (t ha^{-1})	6.31 ± 0.13^{a}	5.41 ± 0.10^{b}	2.46 ± 0.24^{c}	6.16 ± 0.63^{ab}
Below-ground dry biomass (t ha^{-1})	0.80 ± 0.07^{a}	0.90 ± 0.09^{a}	0.54 ± 0.09^{b}	0.68 ± 0.09^{b}
HI (%)	32.66 ± 0.94^{a}	30.01 ± 0.30^{a}	34.05 ± 0.98^{a}	34.04 ± 1.00^{a}
2013-2014				
Plant density (n. m^{-2})	273.6 ± 32.7^{b}	582.5 ± 68.5^{a}	234.7 ± 13.6^{b}	595.9 ± 61.6^{a}
Plant height (cm)	92.0 ± 0.8^{a}	75.0 ± 12.0^{b}	92.3 ± 6.2^{a}	68.0 ± 7.2^{b}
Plant winter survival (%)	98.1 ± 0.66^{a}	80.0 ± 4.9^{b}	82.4 ± 7.2^{b}	61.6 ± 5.3^{c}
Potential seed yield (t ha^{-1})	4.67 ± 0.18^{a}	2.14 ± 0.06^{b}	1.81 ± 0.22^{c}	1.45 ± 0.15^{d}
Actual seed yield (t ha^{-1})°	1.59	1.81	0.74	0.75
Seed moisture at harvest (%)	7.52 ± 0.28^{c}	6.20 ± 0.10^{d}	8.10 ± 0.11^{b}	8.41 ± 0.11^{a}
Num. of capsules per m^2	6634.8 ± 685.0^{b}	7800.0 ± 512.0^{a}	6137.4 ± 808.2^{b}	4887.2 ± 400.1^{c}
Num. of seeds per capsule	10.17 ± 1.04^{a}	4.15 ± 0.38^{b}	4.21 ± 0.54^{b}	4.29 ± 0.56^{b}
1000-seeds weight (g)	6.92 ± 0.10^{a}	6.60 ± 0.11^{b}	7.00 ± 0.08^{a}	6.92 ± 0.10^{a}
Above-ground dry biomass (t ha^{-1})	11.07 ± 0.27^{a}	5.11 ± 0.63^{b}	4.50 ± 0.44^{c}	4.79 ± 0.20^{bc}
Below-ground dry biomass (t ha^{-1})	0.89 ± 0.14^{a}	0.85 ± 0.01^{a}	0.90 ± 0.10^{a}	0.65 ± 0.10^{a}
HI (%)	29.67 ± 1.12^{a}	29.52 ± 1.91^{a}	28.68 ± 0.87^{a}	23.25 ± 0.89^{b}

°Actual seed yield is obtained by mechanical harvest of the entire field plot. a-dFor each year, values in each row followed by the same letter are not significantly different at the 0.05 level using least significance difference test. HI, harvest index.

(1.59 and 1.81 t ha^{-1}, in Pisa and Bologna, respectively) (Table 4). This confirmed the better adaptability of Sideral to the cultivation sites here tested, in comparison with Buenos Aires. The ratios between actual and potential (estimated) seed yields were about 40 and 44% for Sideral and Buenos Aires in Pisa, and 62 and 38% for Sideral and Buenos Aires in Bologna, as mean values over the 2 years.

As general trend, our results showed as higher plant densities decreased the seed yield, in turn decreasing the number of capsules per square meter and the number of seed per capsule, according to previous reports (Casa *et al*., 1999). In fact, it was possible to observe as, in Pisa, the plant density for both genotypes decreased from the 1st to 2nd growing season while the number of capsule per square meter increased and, consequently, a higher seed yield was obtained. Similarly, in northern environment, a lower plant density for Buenos Aires, due to the low cold resistance of this variety, was observed in the 1st year in comparison with the following season, accompanied by a higher number of capsules per square meter and, consequently, by a higher seed yield, compared to 2013-2014. Previous reports (Diepenbrock and Porksen, 1993) have demonstrated as, below a minimum stand density of 400 seeds m^{-2}, compensation by means of apical and basal branches was considered ineffective to reach the equilibrium level of production. In addition, our results were in agreement with previous reports (Rashid *et al*., 1998; Lafond, 2001; Lafond *et al*., 2008), confirming that the three major yield components in linseed are number of capsule per unit area, number of seeds per capsule and seed weight.

Regarding above-ground dry biomass (lignocellulosic residues), in the 1st year of cultivation, Sideral grown under the pedo-climatic conditions of central Italy, and Buenos Aires grown in Bologna gave similar yields in both seasons, while in the 2nd growing season the highest ligno-cellulosic residues were obtained by Sideral cultivated in Pisa. No significant differences were observed for the other ones (Table 4). In relation to the harvest index, in 2012-2013 no significant differences were recorded between the two genotypes in the two locations (32.7% as mean value). As general trend, in the second year of trial a drop in HI values was recorded (27.8% as mean value), with the lowest value for Buenos Aires grown in Bologna. The reduction in HI observed in the 2013-2014 trial can be mainly due to a reduction of plant number per square meter which was accompanied by an increase of above-ground biomass, negatively influencing the HI. This behaviour was particularly evident in central Italy. In addition, in the northern conditions, in 2013-2014 trial a varietal effect on HI was also registered. However, the HI values observed in this study were in accordance with previous findings (Casa *et al*., 1999; Berti *et al*., 2010). Finally, in the 1st year of cultivation a significant effect of genotype was observed for the belowground biomass, with highest value recorded in Sideral, which produced a more developed root systems than Buenos Aires, in both locations according to its best establishment. On the contrary, in the second year no differences were detected.

The obtained results underlined that there was a high variability in the yield response of linseed due to varietal effects and pedo-climatic conditions of cultivation site (in particular temperature levels, and moisture regimes), confirming as yield and yield components are quantitatively inherited and influenced by genotype, environment (location and climate) and their interactions (Gubbels, 1978; Diepenbrock *et al*., 1995; Cross *et al*., 2003, Berti *et al*., 2010). Genotype × environment interaction (G×E) results because individual genotypes differ in their responses to variations in soil fertility, soil moisture, temperature, day-length, light intensity, humidity, disease, cultural practices and other environmental factors (Poehlman, 1987; Basford and Cooper, 1998). G×E reduces association between phenotypic and genotypic values (Romagosa and Fox, 1993), and may cause selections from one environment to perform poorly in another (Adugna and Labuschagne, 2003). Measurement of G×E is also important to determine an optimum breeding strategy for releasing genotypes with adequate adaptation to their target environments (Adugna and Labuschagne, 2003).

Seed chemical composition and oil yield

The analysis of seed chemical composition showed that oil content was strongly dependent on the cultivar x location interaction (Table 5). In both years of cultivation, the highest value was recorded for Buenos Aires, grown in central Italy. It is reported as linseed oil content ranges between 38 and 45% depending on geographical area, cultivars, and environmental conditions (Oomah and Mazza, 1995; Daun *et al*., 2003). Our results are consistent and in some cases higher with those reported in literature (Daun *et al*., 2003; Lafond *et al*., 2008; Dordas, 2010) and underlined the good oil percentage of these linseed varieties that ranged between 44 and 49 % on dry matter.

According to the corresponding seed yields (being the oil yield the product of seed yield and oil content), in the 1st year of cultivation the highest oil yield was recorded for Sideral grown in Pisa and for Buenos Aires grown in Bologna (Table 5). In the 2nd season, Sideral cultivated in Pisa gave the best oil yields, followed by Sideral grown in Bologna. In addition, an opposite varietal effect can also be observed in Bologna, along the two growing seasons, with Buenos Aires as the most produc-

Table 5. Seed oil and protein contents (% on dry matter basis) and oil yield (kg ha^{-1}) (results are the means ± standard deviation of four replicates) in the two linseed varieties grown in the central (Pisa) and northern (Bologna) Italy, during two consecutive growing seasons.

	Sideral		Buenos Aires	
	Pisa	Bologna	Pisa	Bologna
2012-2013				
Seed oil content (%)	46.3±0.2^{b}	43.7±0.2^{d}	48.7±0.5^{a}	44.7±0.1^{c}
Oil yield (kg ha^{-1})	1416.8±98.1^{a}	1014.5±76.2^{b}	620.7±41.2^{c}	1419.0±94.6^{a}
Crude protein content (%)	16.8±1.4^{b}	24.8±0.1^{a}	17.4±0.7^{b}	25.0±0.6^{a}
2013-2014				
Seed oil content (%)	45.2±1.6^{b}	45.8±0.6^{b}	47.4±1.10^{a}	45.8±0.1^{b}
Oil yield (kg ha^{-1})	2110.8±85.6^{a}	977.8±27.5^{b}	858.7±65.9^{c}	662.2±69.6^{d}
Crude protein content (%)	21.3±1.8^{a}	21.3±0.1^{a}	18.2±0.4^{b}	21.3±0.4^{a}

$^{a-d}$For each year, values in the row followed by the same letter are not significantly different at the 0.05 level using least significance difference test.

tive in 2012-2013 and Sideral in 2013-2014. Finally, in the 1st year, the crude protein content was significantly higher in the two genotypes grown in Bologna in comparison with those cultivated in Pisa, while, in the following year, only Buenos Aires grown in Pisa showed the lowest protein content. As general observation, the crude protein content was negatively correlated with oil content (Figure 2) confirming that protein content in seeds depends on their oil content: the higher its amount, the lower the protein content (Bhatty and Cherdkiatgumchai, 1990; Saastamoinen *et al*., 2013).

From the analysis of fatty acid composition (Table 6), linseed oils from the two varieties were characterized by a very stable proportion of polyunsaturated fatty acids with a high content of alpha-linolenic acid (more than 57%) that is the most important fatty acid in linseed. For the high content of this fatty acid, linseed oil is highly reactive and oxidatively unstable and, consequently, mainly used in paints, resins, varnishes, printing inks, linoleum, and more recently, in polymer and oleochemical products, such as epoxidised linseed oils for plastic formulation (Hill, 2000; Salimon *et al*., 2012; Samarth and Mahanwar, 2015). It is also being investigated for use in the building and construction industry (Desroches *et al*., 2012).

Buenos Aires showed, in both years and environments, a significantly higher content of around 5% of linolenic acid, while Sideral was characterized by a higher content of linoleic and oleic acid than Buenos Aires. The climatic differences among environments and years did not significantly influence oil composition, confirming previous reports (Berti *et al.*, 2010). So, averaging the values over year and environment, no significant differences were observed between genotypes for saturated fatty acids (SFA) (Figure 3). On the other hand, Buenos Aires oil showed a highest polyunsaturated fatty acid (PUFA) content (+2.7%), while Sideral oil was characterised by the highest monounsaturated fatty acid content (+13.3%). The PUFA/SFA ratio ranged from 7.99 (for Sideral) to 8.65 (for Buenos Aires).

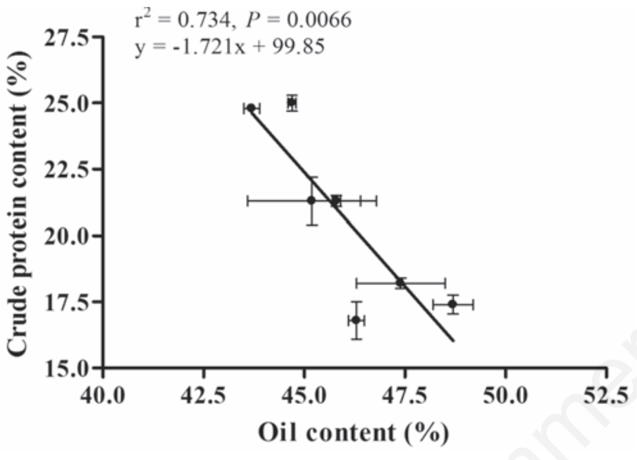

Figure 2. Relationship between crude protein content (%) and oil content (%) measured during the two growing seasons for both genotypes and environments. Vertical and horizontal bars indicate standard deviations from the means of the crude protein content and oil content, respectively.

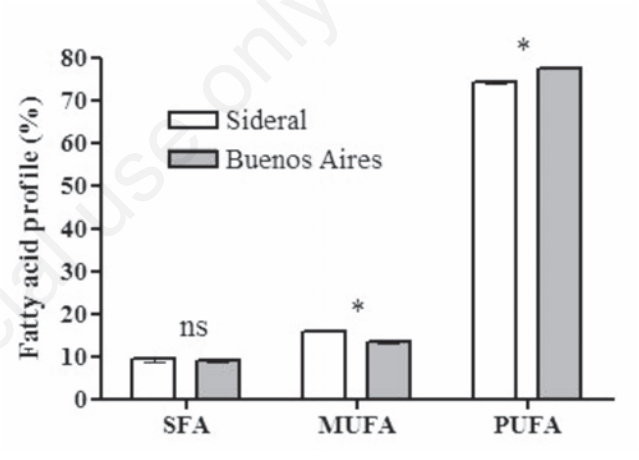

Figure 3. Total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in Sideral and Buenos Aires oils, as mean value over year and environment. ns, not significant; *significant at P≥0.05.

Table 6. Fatty acid composition (results are the means ± standard deviation of four replicates) of the two linseed varieties (Sideral and Buenos Aires) grown in the central (Pisa) and northern (Bologna) Italy, during two consecutive growing seasons.

	Sideral		Buenos Aires	
	Pisa	Bologna	Pisa	Bologna
2012-2013				
C16:0 (palmitic acid)	5.7 ± 0.1^{b}	5.6 ± 0.0^{b}	6.1 ± 0.1^{a}	6.1 ± 0.0^{a}
C18:0 (stearic acid)	3.3 ± 0.1^{a}	3.3 ± 0.1^{a}	2.1 ± 0.1^{c}	2.7 ± 0.0^{b}
C18:1 c9 (oleic acid)	13.3 ± 0.1^{b}	16.1 ± 0.2^{a}	12.1 ± 0.1^{c}	13.4 ± 0.4^{b}
C18:2 c9.c12 (linoleic acid)	18.2 ± 0.2^{a}	15.3 ± 0.1^{b}	13.2 ± 0.1^{c}	12.6 ± 0.1^{d}
C18:3 c9.c12.c15 (linolenic acid)	57.4 ± 0.2^{d}	59.2 ± 0.1^{c}	62.1 ± 0.5^{b}	64.9 ± 0.6^{a}
2013-2014				
C16:0 (palmitic acid)	6.4 ± 0.1^{b}	5.9 ± 0.0^{c}	6.7 ± 0.1^{a}	6.3 ± 0.1^{b}
C18:0 (stearic acid)	3.4 ± 0.1^{b}	3.9 ± 0.1^{a}	2.6 ± 0.1^{d}	3.0 ± 0.0^{c}
C18:1 c9 (oleic acid)	13.9 ± 0.7^{b}	15.7 ± 0.0^{a}	12.1 ± 0.1^{c}	13.2 ± 0.1^{b}
C18:2 c9.c12 (linoleic acid)	16.2 ± 0.3^{a}	15.6 ± 0.1^{b}	13.7 ± 0.4^{c}	12.8 ± 0.2^{d}
C18:3 c9.c12.c15 (linolenic acid)	59.3 ± 1.1^{b}	58.4 ± 0.1^{b}	64.3 ± 0.8^{a}	64.2 ± 0.1^{a}

$^{a-d}$For each year, values in the row followed by the same letter are not significantly different at the 0.05 level using least significance difference test.

Above- and below-ground biomasses characterisation

In Table 7, moisture at harvest, nitrogen and carbon content, C/N ratio as well as the potential C and N additions by above- and below-ground biomasses were reported. Our results pointed out as epigeal and hypogeal residues were different in terms of quantity, chemical characteristics and, consequently, timing and conditions of decomposition, depending on cultivar and environment (Table 3). Hypothesizing a full return to the soil of above-ground biomass after seed harvesting, Sideral grown in Pisa in both growing seasons, and Buenos Aires cultivated in Bologna during the 1st year, could provide to the soil major amounts of N and C in comparison with the other ones. In the 1st year, the C/N ratio for lignocellulosic residues was higher in Bologna for both varieties, in comparison with that observed in Pisa. On the contrary, in the 2nd year, the highest ratio in Sideral grown in Pisa was recorded.

The potential root contribution in N and C supply varied between genotypes and environment, according to the corresponding measured yield. As general behaviour, Sideral, in both environments, contributed to a higher N and C supply to the soil, compared to Buenos Aires. On the contrary, a greater variability of C/N ratio in the case of roots between genotypes and environments has been noted. The C/N ratio, varied from 40 to 160, similarly to a wide range of common crop residues, and the decomposition of these high C:N ratio residues actually withdraws nitrogen from the soil, temporarily immobilizing the nutrient during the early stages of decay and thereby reducing the short-term productivity of the soil (Smill, 1999).

Table 7. Chemical composition and nutrient additions (results are the means ± standard deviation of four replicates) by above- and below-ground biomass of the two linseed varieties (Sideral and Buenos Aires) grown in the central (Pisa) and northern (Bologna) Italy, during two consecutive growing seasons.

	Sideral		Buenos Aires	
	Pisa	Bologna	Pisa	Bologna
Above-ground biomass 2012-2013				
Yield (t ha^{-1})	6.31±0.13^{a}	5.41±0.10^{b}	2.46±0.24^{c}	6.16±0.63^{ab}
Moisture at harvest (%)	7.0±0.1^{ab}	7.8±0.9^{a}	6.9±0.4^{b}	5.7±0.7^{c}
C (%)	44.74±0.23^{c}	44.20±0.11^{c}	48.73±0.07^{a}	45.71±0.40^{b}
N (%)	0.65±0.02^{b}	0.46±0.01^{c}	0.80±0.01^{a}	0.50±0.06^{c}
C/N	68.8±8.5^{b}	96.1±9.8^{a}	60.9±7.0^{b}	91.4±6.7^{a}
kg N ha^{-1}	41.0±0.8^{a}	24.9±0.1^{c}	19.7±0.2^{d}	30.8±1.1^{b}
kg C ha^{-1}	2823.9±55.9^{a}	2391.2±49.9^{b}	1198.8±43.5^{c}	2815.7±38.6^{a}
2013-2014				
Yield (t ha^{-1})	11.07±0.27^{a}	5.11±0.63^{b}	4.50±0.44^{c}	4.79±0.20^{bc}
Moisture at harvest (%)	11.1±1.6^{bc}	17.1±1.4^{a}	9.2±0.4^{c}	12.1±0.9^{b}
C (%)	49.82±0.41^{b}	51.90±0.11^{a}	51.62±0.11^{a}	51.80±3.70^{a}
N (%)	0.42±0.05^{c}	0.70±0.06^{b}	0.84±0.01^{a}	0.70±0.10^{b}
C/N	118.6±8.2^{a}	74.1±1.7^{b}	61.5±9.0^{b}	74.0±3.7^{b}
kg N ha^{-1}	46.5±1.2^{a}	35.8±2.1^{b}	37.8±0.4^{b}	33.5±1.3^{b}
kg C ha^{-1}	5515.1±135.9^{a}	2652.1±167.0^{b}	2322.9±94.5^{c}	2481.2±105.4^{bc}
Below-ground biomass 2012-2013				
Yield (t ha^{-1})	0.80±0.07^{a}	0.90±0.09^{a}	0.54±0.09^{a}	0.68±0.09^{a}
Moisture at harvest (%)	7.5±0.1^{b}	8.5±0.4^{b}	12.1±2.1^{a}	7.7±0.4^{b}
C (%)	41.38±0.34^{c}	51.90±0.11^{a}	45.41±0.52^{b}	45.61±0.20^{b}
N (%)	1.05±0.02^{a}	0.62±0.01^{c}	0.80±0.02^{b}	0.60±0.01^{c}
C/N	39.4±4.2^{d}	83.7±6.3^{a}	56.8±7.1^{c}	76.0±6.2^{b}
kg N ha^{-1}	8.4±0.7^{a}	5.6±0.8^{b}	4.3±0.7^{c}	4.1±0.5^{c}
kg C ha^{-1}	331.0±27.6^{b}	467.1±50.0^{a}	245.2±38.7^{c}	310.1±25.3^{b}
2013-2014				
Yield (t ha^{-1})	0.89±0.14^{a}	0.85±0.01^{a}	0.90±0.10^{a}	0.65±0.10^{a}
Moisture at harvest (%)	21.1±3.3^{b}	16.0±0.1^{c}	31.8±2.8^{a}	13.1±0.1^{d}
C (%)	47.81±0.18^{b}	50.39±0.10^{a}	50.25±0.19^{a}	45.20±0.23^{c}
N (%)	0.30±0.06^{d}	1.00±0.09^{a}	0.43±0.08^{c}	0.88±0.02^{b}
C/N	159.3±21.3^{a}	50.6±4.9^{c}	116.9±20.1^{b}	51.4±6.0^{c}
kg N ha^{-1}	2.7±0.7^{d}	8.5±0.3^{a}	3.9±0.3^{b}	5.7±0.9^{c}
kg C ha^{-1}	424.3±84.4^{a}	428.3±65.7^{a}	452.25±31.1^{a}	293.8±89.1^{b}

C, carbon; N, nitrogen. $^{a\text{-}d}$For each year, values in the row followed by the same letter are not significantly different at the 0.05 level using least significance difference test.

These characteristics are very important in the prevision to incorporate the lignocellulosic biomass into the soil, since a significant quantity of nutrients (N and C) might hypothetically return to the soil each year by above- and below-ground biomass. Linseed residual biomasses can be involved in the organic matter and nutrients cycling in the soil (stems and roots), thanks to their chemical composition. In this way, a part of removed nutrients could be returned into the soil by the stem portions after seed harvest, and also by the roots, which remain directly in the soil and can contribute to the build-up of the soil organic matter pool. The incorporation of high quality residual biomass makes carbon more readily available for nutrient cycling and captures more carbon through soil biotic processes that lead to reduced CO$_2$ flux, and improves soil physical properties that may enhance both short- and long-term productivity (Guo *et al*., 2009).

Conclusions

This study underlined the possibility to successfully grow linseed in the pedoclimatic conditions of central and northern Italy. The obtained results confirmed as, in linseed, yield and yield components, as well as oil content and composition and chemical characteristics of epigeal and hypogeal residues, are quantitatively inherited and influenced by both genotype and environment (location and climate). A wide range of variation across years and locations, mainly due to environmental factors such as temperature levels and moisture regimes, have been recorded. As expected, in fact, the climatic differences between the two environments and, for the same environment, between the two years, significantly influenced the investigated parameters, confirming as climate and location, together to genotype, played a key role in defining the quanti-qualitative characteristics in linseed production. In particular, in northern environment, characterised by a continental climate, a careful choice of the varieties for their resistance to cold should be considered. Finally, this experience confirmed the possibility of linseed cultivation with reduced input as a starting point for crop sustainability.

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