

Article

Soil Fertilization with Urea Has Little Effect on Seed Quality but Reduces Soil N₂O Emissions from a Hemp Cultivation

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Abstract: Multipurpose production of hemp has led to renewed interest for this crop cultivation, especially for human nutrition. To date, no information about the influence of nitrogen source on hemp seed quality is available. Hemp is also used for food and beverages due to its nutritional characteristics. This further use of hemp has led to an increase in hemp-grown areas. Therefore, it is important to get more information on the role of nitrogen on the quality production as well as to evaluate the environmental impact of the cultivation technique. In this work, we evaluate the influence of nitrogen source (i.e., NH₄NO₃ and urea) on the seed fatty acid composition of an edible hemp as well as on the environment in terms of soil N₂O emission. Nitrogen source modified seed quality very little. Even if characterized by a lower acidic profile, seed from plants grown under urea and NH₄NO₃ had a ω-3/ω-6 ratio (0.3) within the optimal range from the nutritional standpoint, being considered as the optimal proportion for human metabolism and health. Urea fertilization reduced soil N₂O emission. Our findings suggest that nitrogen source seems not to influence seed quality and that urea fertilizer might be more climate-friendly than NH₄NO₃ in terms of greenhouse gas emissions, in an extensive cultivation of hemp for industrial use.

Keywords: soil nitrogen fertilization; fatty acid profile; N₂O emission; *Cannabis sativa* L.

1. Introduction

Multipurpose production of hemp (*Cannabis sativa* L.) has led to renewed interest for this crop cultivation, especially for human nutrition. Recently, many countries have reintroduced hemp cultivation for industrial use by using varieties of *C. sativa* characterized by low tetrahydrocannabinol (THC) levels. There are many products obtained from industrial hemp and they have multiple uses in

different fields, such as agriculture, food, textiles, construction, bio-composites, papermaking, biofuel, cosmetics, and personal care [1,2]. For newly introduced crops such as hemp, it is necessary to assess the modern production technology which is most appropriated to the growing environment. It is essential to study the adaptability of the different variety in the different environment, in particular in the Mediterranean environment, due to the North Europe cultivar origin, with the focus of agronomic techniques supporting multipurpose hemp production [3]. The agricultural system plays a central role in sustainable development. Its fundamental position as a supplier of human nutrition shapes the global economy. It is central to achieving a suite of sustainable development goals, ranging from ending hunger to improving human well-being and reducing environmental impacts. Therefore, the agronomic technique applied should minimize the environmental impact for the future generation [4]. Hemp is a low-input plant, but the nitrogen (N) has to be supplied in the right quantity; in fact, a surplus of N has a negative effect on growth and fiber quality, which have effects on the crop yield [5]. Nowadays, the growing interest in hemp, and therefore the increase of its cultivation, could have a negative impact on the global environment and climate in terms of greenhouse gases' (GHGs) production due to the use of fertilizers to enhance biomass yield. Therefore, it is essential to define a suitable agronomic governance in order to intensify hemp cultivation in a sustainable manner, with a minimal impact on environment and climate. Such crop management should also guarantee, at the same time, elevated yields and quality in terms of biomass, seeds, or fibers. A few recent studies have evaluated the performance of different hemp cultivars on seed yield and quality [6,7] or the influence of seed storage on seed quality [8], as well as the impact of fertilization of greenhouse gas emissions [9]. However, the above-mentioned studies did not concurrently address the issue of how crop management affects biomass production, seed quality, and environment and climate (i.e., soil GHG emissions). In particular, some studies [9] were carried out, aimed at optimizing the benefits of hemp as an energy crop, by identifying the nitrogen fertilization levels, which minimize GHG emissions and optimize energy inputs. In these studies, the authors did not perform direct GHG measurements, but they used a simple approach to calculate nitrous oxide emissions, based either on the Intergovernmental Panel on Climate Change (IPCC) methodology [10] or on an exponential function relating N inputs to N₂O emission [11]. It is well known that GHG emissions are also largely affected by crop management in terms of nitrogen source and time of fertilizer application and irrigation [12]. The soil fertilization and in particular the nitrogen enrichment plays an important role in plant growth and yield. Although the effect of nitrogen rates on hemp yield has been studied by a number of researchers [9,13,14], previous studies did not analyze the effect of nitrogen source on yield and chemical composition of seeds as well as on soil N₂O emissions [4]. In a hemp crop, the supply to soil of N fertilizers mainly occurs at sowing and under this circumstance, the biological transformations and meteorological conditions might favor a N loss from soil, thereby reducing the effectiveness by which plants utilize the fertilizer during the vegetative growth. The use of fertilizers characterized by a slow N release such as urea should be, indeed, advantageous for plants that should efficiently use the fertilizer, thereby subtracting nitrogen to microbes involved in soil N₂O production [15,16]. On the contrary, mineral fertilizers such as the ammonium nitrate that make nitrogen immediately available in the soil are quickly depleted in the soil, due to the fast NH₄⁺ oxidation by nitrifying bacteria producing N₂O and due to NO₃⁻ lost by leaching. The depletion of nutrients could seriously effect productivity of plants and the available nitrogen might limit the yield and alter the chemical composition of seeds. Information about how the nitrogen source might affect biomass production, seed quality, and soil GHG emissions is actually missing for hemp cultivation [4], and this knowledge gap needs be filled because of increasing interest for multipurpose production of hemp.

The main objective of this study was to investigate the effect of different nitrogen sources (i.e., ammonium nitrate and urea) on chemical composition of hemp seeds as well as on soil N₂O emissions. More specifically, we hypothesized that, as compared to ammonium nitrate, the relatively fast urea mineralization improves the N availability for plants over time, enhances the plant yield and seed quality and, at the same time, mitigates N₂O emission from soil.

2. Materials and Methods

2.1. Experimental Site, Plant Material, and Crop Management

The field trial was carried out during April–July 2017 at a flat agricultural site inside an urban agglomeration in the suburbs of Naples (Italy) (50 m a.s.l.; 40°86′ N, 14°33′ E), characterized by Mediterranean climate conditions with warm dry summer and mild wet winter (Figure 1).

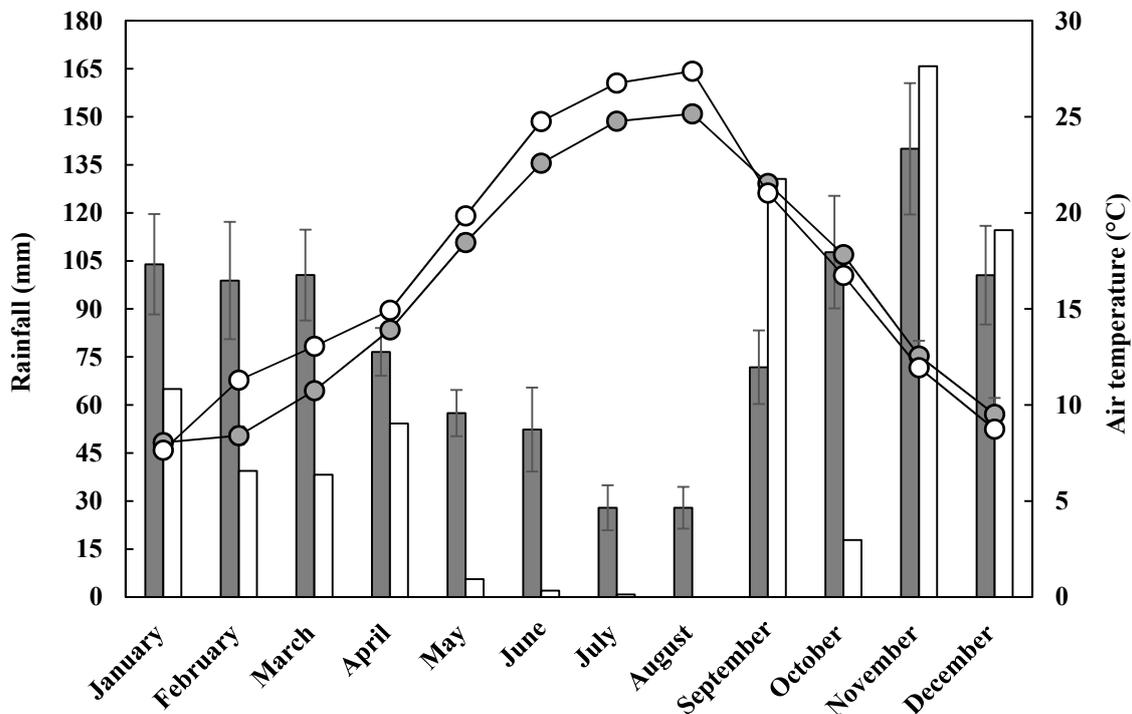


Figure 1. Monthly air temperature (circles) and rainfall (bars). Grey: mean 1996–2016 values; white: 2017 values.

The soil has a sandy-loam texture and the relative content of the different fractions for the 0–0.1 m soil layer is: sand 80%, silt 12%, and clay 8%, bulk density is equal to 1.37 g cm⁻³. Chemical characteristics for the 0–0.1 m layer are: 2.54% organic matter content, absence of total carbonate, electrical conductivity of soil 0.149 dS m⁻¹, and pH_(H₂O) 7.08. The soil properties were determined as described by Dane and Topp [17] and Sparks et al. [18]. The site was equipped with a meteorological station (Rotronic MP100, Campbell Scientific Ltd, Shepshed, UK) that monitored air temperature and humidity plus rainfall by means of a rain gauge.

Cannabis sativa L. cv. Wojko (Poland), a dual-purpose monoecious variety for fiber and seed production, was sown on 19 April 2017 at a spacing of 0.4 × 0.2 m. The experimental design was a randomized block with two nitrogen (N) fertilization treatments, i.e., ammonium nitrate (NH₄NO₃, 26% N) and urea (46% N), and each N treatment included four replicates consisting in 9 m² plots. In this study—in which the goal was to evaluate the effect of N source on hemp seed quality and on soil GHG emissions—it was purposely missing a control plot with no fertilizer (N = 0). The control seeds (CTR) later reported in the text were used to indicate seeds purchased by the seed company, used for the sowing in our experiment, and utilized as a standard control in seed quality analysis.

Hemp was sown at a nominal density of 45 kg seeds ha⁻¹ and an area of 1 m² was kept undisturbed for final harvest that occurred on 27 July. A total of 80 kg ha⁻¹ N from NH₄NO₃ or urea was supplied to plots at sowing by uniformly and manually spreading the fertilizer on the soil and incorporating it into the soil with a rake. Based on recommendations for hemp production—the N fertilization for hemp should be between 60 and 120 kg ha⁻¹ N [19]—we decided to apply 80 kg ha⁻¹ N. This quantity was

chosen taking into account the soil type present, with the idea of making a quantity of nitrogen useful for the plant and sustainable, regardless of the nitrogen source, for the GHG emissions that could be generated. Two sprinkler irrigations at a total of 21 mm were applied after sowing to guarantee a uniform plant emergency. All plots were watered 5 times during the growth cycle until 27 June (69 days after sowing, DAS) receiving a total seasonal water volume of 827 m³ ha⁻¹. No weeding occurred before or during the growth season, but weeds were eliminated manually if necessary.

2.2. Seed Proximate Composition

Proximate composition (moisture, crude protein, crude fat, and total ash) of seeds was determined using the Official Methods of Analysis of Association of Official Analytical Chemists (AOAC) International [20]. Total carbohydrates were determined by difference: 100% – (crude protein + crude fat + total ash + moisture)%.

2.3. Fatty Acid Methyl Esters (FAMES) Preparation

The fatty acid profile was determined by gas chromatography, as fatty acid methyl esters (FAMES) by gas chromatographic analysis, according to Siano et al. [7]. The extract seed oil was transferred to a pyrex test tube with a screw cap, and 2 mL of methanolic-HCl solution 1.25 M was added. The sample was then placed in a water bath at 90 °C for 60 min. The fatty acid methyl esters were extracted with *n*-hexane, after the addition of distilled water. The solution was filtered using Millex 0.45 µm polyvinylidene fluoride (PVDF) disposable syringe filters (EMD Millipore Corp., Billerica, MA, USA) and 1 µL was directly injected into the gas chromatograph for analysis.

2.4. FAMES Gas Chromatographic Analysis

The fatty acid content of samples was determined using a SP-2560 (100 m × 0.25 mm × 0.2 µm, Supelco, Inc., Bellefonte, PA, USA) capillary column [7]. Samples were introduced by the injection system split-splitless in split mode (ratio 1:100). The oven temperature program started at 140 °C (held for 5 min), linearly increased to 260 °C (4 °C/min), and was kept at this temperature for the remaining time of analysis. Fatty acids composition was obtained by comparison with retention times of the standard mixture FAMES and expressed as percentage. Data were recorded and processed by the ChromQuest 5.0 software (Thermo Fisher Scientific, Rodano, Italy).

2.5. Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) Spectra

The ATR-FTIR spectroscopy was used to verify the identity, authenticity, and purity of the raw materials and ingredients used in the food industry [21–23] because it provides an immediate semi-quantitative evaluation of macronutrients of analyzed samples. This approach is based on the vibrations of functional groups and highly polar bonds in the components analyzed. ATR-FTIR analyses were performed using a Spectrum 400 spectrophotometer (PerkinElmer, Inc., Waltham, MA, USA), equipped with a deuterated triglycine sulfate (DTGS) detector. Overall, 32 scans/spectrum were acquired in the 4000–650 cm⁻¹ range, with a resolution of 4 cm⁻¹. Samples were analyzed without any previous treatment. In order to test the repeatability, analyses were performed in triplicate and average spectra were used. Spectra were elaborated by using the PE Spectrum software version 10.5.1 (PerkinElmer, Inc., Waltham, MA, USA).

2.6. Biometrical Determinations

Plant harvesting occurred at 99 DAS, when seeds were mature, by collecting plants from a 1 m² surface per plot. Plants were separated into shoots and roots and dried in an oven until constant weight. At the same time (99 DAS), seeds were collected, air dried, and used for chemical analysis.

Oil extraction from seeds was performed by the solvent extraction method by using a Soxhlet apparatus. In particular, a sample of 20 g of ground seed was extracted with the diethyl ether as a

solvent, for 6 h. The solvent was removed with a rotary evaporator (Mod. Hei-VAP Value, Heidolph Instruments GmbH & CO. KG, Schwabach, Germany) at 40 °C. The residue was placed in a drier and weighed up to constant value. The resultant oil was flushed with nitrogen and stored at −20 °C until further analysis.

Nitrogen use efficiency (NUE) of the crop was calculated as the ratio between total biomass production and applied N (kg kg^{−1} N).

2.7. Soil and Gas Measurements

The monitoring of soil N₂O emissions, performed two times during the day (07:00–12:00 and 13:30–18:30 solar time), was carried out from 2 to 86 DAS by using 0.3 m diameter and 0.1 m high autochambers inserted 0.03 m deep into the soil. We used one chamber per plot and applied the following protocol: for each chamber, air samples were automatically sampled before and three times during the closing of the lid of the chamber and transferred by means of a 15 m long Teflon tube to a gas chromatograph (SRI 8610C, SRI Instruments, Torrance, CA, USA), equipped with an electron capture detector (ECD). Gas standards were analyzed before and after each measurement was run. N₂O fluxes (F_{N_2O}) were calculated as:

$$F_{N_2O} (\mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}) = PV (AT_aR)^{-1} dCdt^{-1} \quad (1)$$

where P is atmospheric pressure, V is the chamber volume, dC/dt is the slope of the gas concentration changing curve over time, A is the surface area of the chamber, T_a is the air temperature, and R is the gas constant. A linear or exponential regression model was used to calculate $dCdt^{-1}$, taking into account the R^2 values. Only curves where the regression slope did not change sign over the observation period, i.e., $dC/dt|_{t=0}/dC/dt|_{t=30} > 0$, were considered in the results [15,16]. The N₂O emission factor (EF1) for each fertilizer type was calculated according to IPCC [24] as:

$$EF_1 (\%) = f_c (\text{kg N}_2\text{O-N ha}^{-2})/N (\text{kg ha}^{-2}) \times 100 \quad (2)$$

where f_c is the cumulative flux and N is the nitrogen amount supplied at sowing.

The nitrous oxide emission intensity (NEI)—also known as yield-scaled emission—was calculated as the ratio between N₂O cumulative flux and biomass yield, considering a global warming potential for N₂O of 298-fold greater than that of CO₂ on a 100-year horizon and expressed in CO₂ equivalent as kg CO₂eq kg^{−1}.

In order to determine the soil NO₃[−] content, soil samples were taken before and after the fertilizer application. In the latter case, soil samples were taken twice a week during the first two weeks; then, the samples were taken weekly until about 30 DAS. NO₃[−] content was determined on soil samples collected from the 0–0.1 m soil layer in the close proximity of the autochambers at the end of the daily air sampling cycle of the relative autochamber. In brief, an integrated soil sample per plot was obtained by collecting different soil samples in the close proximity of each autochamber and they were put together. Soil was air-dried and sieved (2 mm). NO₃[−] content was determined in a 2 mol/L KCl soil extract (1:10 *w/v* ratio) [15] and determined by potentiometry using a combined polymer membrane electrode for nitrate (model 6.00510.120, Metrohm AG, Herisau, Switzerland) and a standard solution of nitrate ion.

2.8. Statistical Analysis

Statistical analysis of biometrical, seed composition, and soil N₂O emissions data was performed by means of the Sigma-Plot package (Sigma-Plot 12.2, Systat Software, Inc., San Jose, CA, USA). Differences in biometrical and seed composition data were checked by one-way analysis of variance (ANOVA) followed by the Duncan's test ($p < 0.05$), whereas soil N₂O emissions data were analyzed by one-way ANOVA repeated measurements followed by the Duncan's test ($p < 0.05$). All chemical analyses on the seeds were performed in quadruplicate and the value was expressed as mean ± standard error.

For the ATR-FTIR, statistical analysis was performed by the Spectrum AssureID software (Version 4.x, trademark of PerkinElmer, Inc., Waltham, MA, USA), the spectra were subjected to Soft Independent Modelling of Class Analogy analysis (SIMCA). SIMCA was used as a chemometric approach, which models the variation within the collection of reference spectra. SIMCA develops separate models (so-called disjointed class models or SIMCA hyperboxes) based on principal component analysis (PCA) for each training set category. More specifically, all spectra were subjected to baseline correction and normalization prior to the statistical analysis (PCA) that was used as an unsupervised classification technique, aiming at sorting the spectra into different categories. The scores plot consisting of a projection of the original data onto principal component axes was used to visualize clustering among samples (sample patterns, groupings, or outliers).

3. Results

3.1. Seed Quality

Table 1 shows the results of the chemical analysis performed on hemp seed samples, in terms of moisture, total lipids, total proteins, ashes, and carbohydrates contents. Seeds obtained from plants fertilized with ammonium nitrate had a similar composition with respect to seeds obtained from plants grown with urea. However, it should be noted that the macronutrients values of samples derived from plants fertilized with NH_4NO_3 and urea are much lower than those of control seeds, whereas an opposite trend for ashes and carbohydrates was found.

Table 1. Proximate composition of seeds from hemp plants fertilized by ammonium nitrate (NH_4NO_3) and urea. Data are means ($n = 4$) \pm standard error (SE). CTR, control seeds. * Dry matter of sample.

| | Carbohydrates (%) * | Protein (%) * | Lipids (%) * | Ash (%) * | Moisture (%) |
|--------------------------|-----------------------------|-----------------------------|----------------------------|----------------------------|---------------|
| CTR | 42.8 \pm 2.5 | 22.1 \pm 1.9 | 30.1 \pm 1.6 | 5.0 \pm 0.3 | 6.4 \pm 0.6 |
| NH_4NO_3 | 69.9 \pm 1.7 ^a | 16.2 \pm 1.4 ^a | 6.9 \pm 0.7 ^a | 7.0 \pm 0.6 ^a | 8.1 \pm 1.3 |
| Urea | 72.5 \pm 1.5 ^a | 14.7 \pm 1.3 ^a | 6.6 \pm 0.7 ^a | 6.2 \pm 0.4 ^a | 7.7 \pm 1.2 |

^a denotes significant differences between treatments and control ($p < 0.05$).

Differences among seeds were also evident in the acid profile of the analyzed samples (Table 2). Fatty acid composition was almost similar between the two treatments under comparison, but significant differences were found once again in comparison to control seeds, which were characterized by a lower content of saturated fatty acids (SFA) and a lower content of polyunsaturated fatty acids (PUFA). No significant differences in monounsaturated fatty acids (MUFA) content among seeds was found. The greater PUFA content in control seeds was due to the higher concentration in linoleic and especially in α -linoleic acid, which determines an elevated value of the ω -3/ ω -6 ratio (i.e., α -linoleic plus γ -linoleic acid/linoleic acid).

Table 2. Fatty acid profile of seeds from hemp plants fertilized by ammonium nitrate (NH₄NO₃) and urea. CTR, control seeds. Data are means (*n* = 4) ± SE.

| Fatty Acid, Area % | CTR | NH ₄ NO ₃ | Urea |
|--------------------------------|--------------|---------------------------------|---------------------------|
| Palmitic, C16:0 | 6.54 ± 0.36 | 11.37 ± 0.77 ^a | 11.9 ± 0.75 ^a |
| Palmitoleic, C16:1 | 0.12 ± 0.03 | 0.22 ± 0.03 ^a | 0.24 ± 0.04 ^a |
| Stearic, C18:0 | 2.69 ± 0.25 | 2.23 ± 0.2 | 2.16 ± 0.28 |
| Oleic, C18:1 ω-9c | 9.85 ± 0.44 | 10.8 ± 0.63 | 10.46 ± 0.38 |
| C18:1 ω-7c | 0.88 ± 0.13 | 1.53 ± 0.21 ^a | 1.61 ± 0.17 ^a |
| Linoleic, C18:2 ω-6c | 55.36 ± 1.68 | 51.12 ± 2.02 ^a | 51.76 ± 1.5 ^a |
| Arachidic, C20:0 | 0.7 ± 0.14 | 1.06 ± 0.28 | 1.03 ± 0.38 |
| γ-Linolenic, C18:3 ω-6 | 3.86 ± 0.46 | 4.55 ± 0.58 | 4.96 ± 0.57 |
| cis-11-Eicosenoic, C20:1 | 0.32 ± 0.09 | 0.51 ± 0.19 | 0.48 ± 0.13 |
| α-Linolenic, C18:3 ω-3 | 17.34 ± 1.35 | 11.1 ± 0.92 ^a | 11.37 ± 1.25 ^a |
| cis-11,14-Eicosadienoic, C20:2 | 1.28 ± 0.27 | 1.31 ± 0.27 | 1.26 ± 0.28 |
| ω3/ω6 Ratio | 0.38 | 0.31 | 0.31 |
| Σ SFA | 9.93 ± 0.46 | 14.66 ± 0.84 | 15.09 ± 0.89 |
| Σ MUFA | 11.17 ± 0.47 | 13.06 ± 0.69 | 12.79 ± 0.44 |
| Σ PUFA | 78.84 ± 2.22 | 68.08 ± 2.31 | 69.35 ± 2.05 |

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. ^a denotes significant differences between treatments and control (*p* < 0.05).

Identification of the functional groups in hemp seed samples was based on the ATR-FTIR peaks attributed to stretching and binding vibrations (Figure 2).

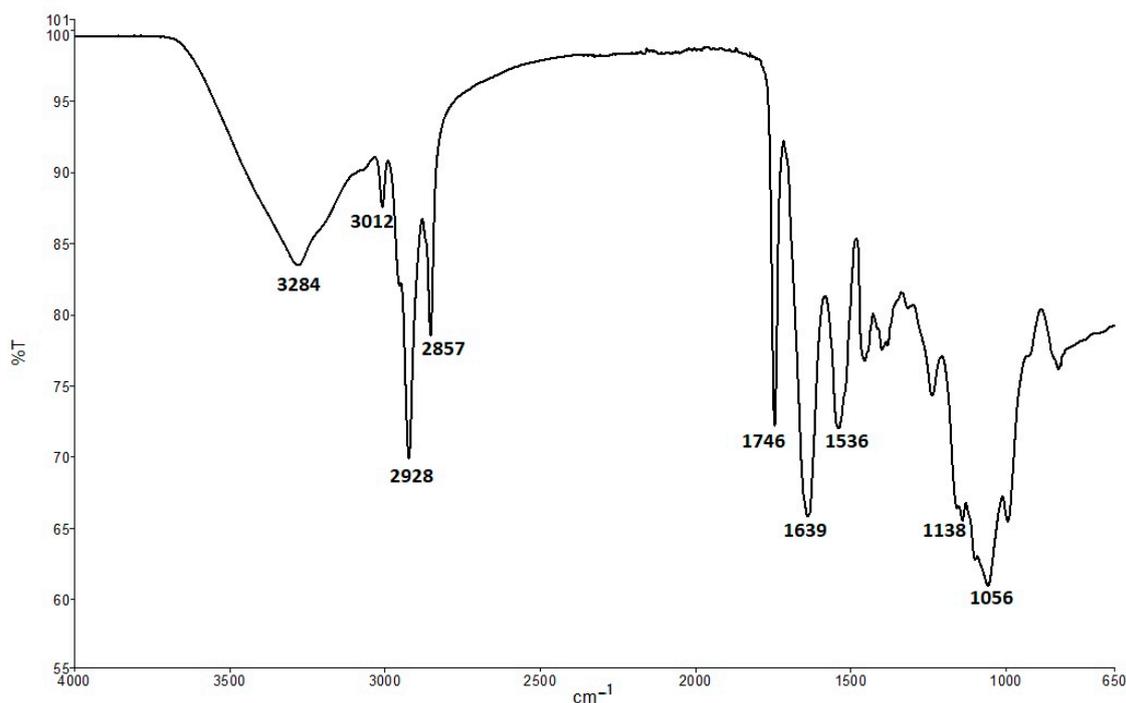


Figure 2. Representative ATR-FTIR absorption spectra in the region 4000–650 cm⁻¹, with the absorptions of principal functional groups, of seeds from hemp plants fertilized by ammonium nitrate (NH₄NO₃) and urea. CTR, control seeds. %T indicates the amount of light that passes through the sample.

The ATR-FTIR spectra of analyzed samples can be divided into different areas corresponding to specific functional groups: area 1150–1000 cm⁻¹ to stretching vibrations by carbohydrates [21]. The area between 1650 and 1500 cm⁻¹ was assigned the O=C-N-H vibrations (region of proteins), in particular to 1639 cm⁻¹, the band of the amide I, and to 1536 cm⁻¹, the band of the amide II [22]. The band to 1746 cm⁻¹ contains vibrations assigned to lipids groups and C=O stretching [23]. Area 2928 cm⁻¹

corresponded to C-H stretching vibrations that were specific to CH₃ and CH₂ and the area centered to 3284 cm⁻¹ corresponded to stretching of the N-H groups.

In order to confirm the suitability of ATR-FTIR spectral data to analyze the effect of soil fertilization on seed quality, we performed a PCA analysis using the SIMCA algorithm (Figure 3). The use of statistical analysis was required to highlight whether the subtle changes observed were statistically significant. Cluster separation was obtained in all ranges corresponding to 4000–650 cm⁻¹, indicating a good separation between groups. According to the SIMCA models, the larger the interclass distance among groups, the better the separation. It is generally accepted that a distance value higher than 3 indicates that the samples are well separated and hence different [25]. In Table 3, the Inter Material Distances of analyzed samples were reported and the obtained result showed a clear separation between control seeds and ammonium nitrate and urea seeds.

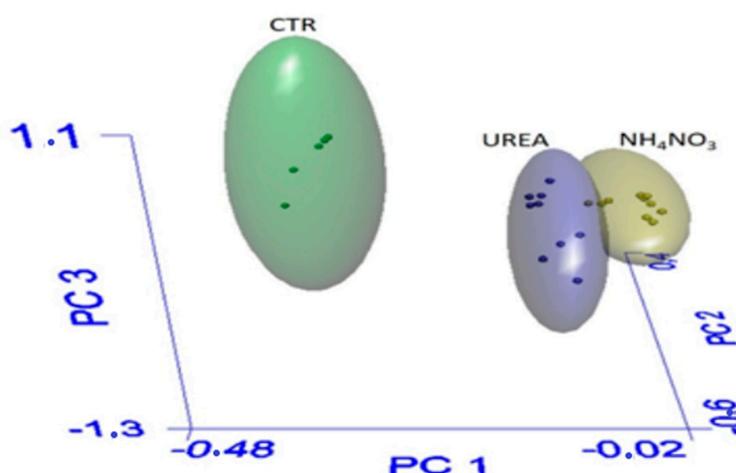


Figure 3. Three-dimensional principal component analysis (PCA) scores plot according with SIMCA algorithm of seeds from hemp plants fertilized by ammonium nitrate (NH₄NO₃) and urea. CTR, control seeds. PC1, PC2, and PC3 are three independent variables.

Table 3. Inter Material Distances.

| Material | CTR | Urea | NH ₄ NO ₃ |
|----------|-----|------|---------------------------------|
| CTR | - | 6.53 | 10.70 |
| Urea | - | - | 3.03 |

3.2. Agronomic Traits and Soil N₂O Emission

NO₃⁻ content in the soil increased after fertilization, which occurred on 19 April 2017. It reached the highest values (about 80 mg kg⁻¹) in all treatments two days after the fertilizer application (2 DAS) and decreased up to about 50 mg kg⁻¹ at 28 DAS. The highest ($p < 0.05$) content of NO₃⁻ in the soil was detected between 14 and 16 DAS in plots fertilized with NH₄NO₃ (Figure 4).

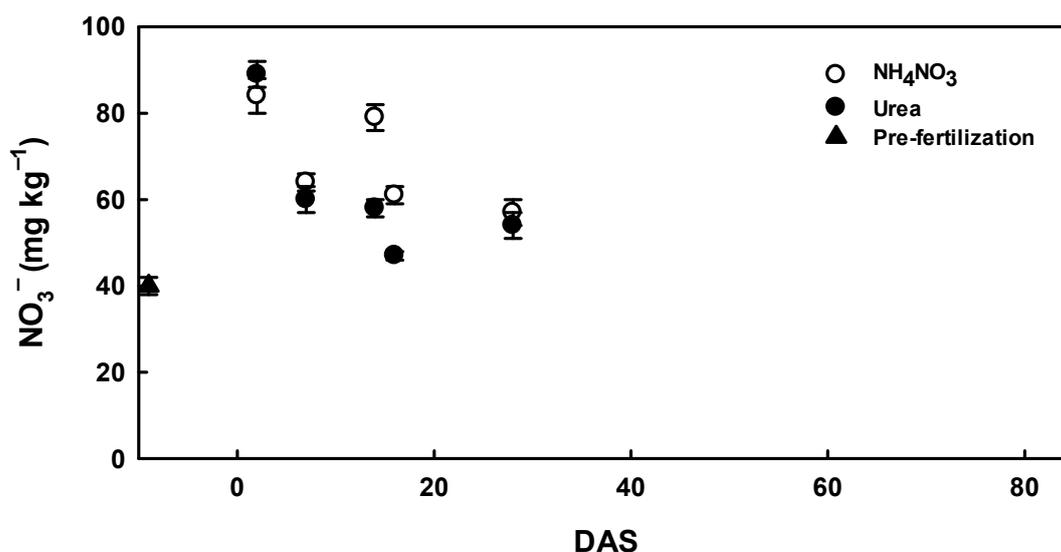


Figure 4. NO₃⁻ content in soil fertilized with ammonium nitrate (open circles) and urea (filled circles). Data are means ($n = 4$) \pm SE.

Hemp plants fertilized with urea produced more biomass ($p < 0.05$) compared to NH₄NO₃ treatment as a result of greater dry matter production both above-ground (shoot) and below-ground (root) (Table 4). The crop fertilized with urea had a greater ($p < 0.05$) nitrogen use efficiency (NUE) with respect to NH₄NO₃ (Table 4).

Table 4. Shoot biomass, root biomass, nitrogen use efficiency (NUE), nitrous oxide emission intensity (NEI), and emission factor (EF) in ammonium nitrate plots and urea plots. Data are means ($n = 4$) \pm SE.

| Treatments | Shoot (g m ⁻²) | Root (g m ⁻²) | NUE (kg kg ⁻¹ N) | NEI (kg CO _{2eq} kg ⁻¹) | EF (%) |
|---------------------------------|-------------------------------|-----------------------------|-----------------------------|--|------------------------------|
| NH ₄ NO ₃ | 775.1 \pm 66.3 ^a | 169.7 \pm 16 ^a | 118 \pm 12 ^a | 0.0053 \pm 0.0006 ^a | 0.2 \pm 0.01 ^a |
| Urea | 979.4 \pm 106 ^b | 232 \pm 26.4 ^b | 151 \pm 16 ^b | 0.0035 \pm 0.0009 ^b | 0.16 \pm 0.03 ^b |

Different letters denote significant differences between treatments. $p < 0.05$.

Overall, N₂O emissions and cumulative N₂O fluxes from the ammonium nitrate treatment were significantly higher ($p < 0.05$) than those fertilized with urea (Figure 5). In both N treatments, the greatest N₂O emission occurred immediately after fertilizer application influenced by irrigation, but the peak in emissions from ammonium nitrate plots was higher (35 μ g m⁻² h⁻¹ and 27 μ g m⁻² h⁻¹, respectively) and was reached some days earlier (Figure 5). Lower peaks of N₂O emission occurred later during the grown season, corresponding to further irrigation events. The higher N₂O emission after 30 DAS determined higher cumulative fluxes in NH₄NO₃ plots compared to urea plots (Figure 5). The nitrous oxide emission intensity (NEI), also known as yield-scaled N₂O emission, and the emission factor (EF₁) resulted lower ($p < 0.05$) in urea treatment than in ammonium nitrate (Table 4).

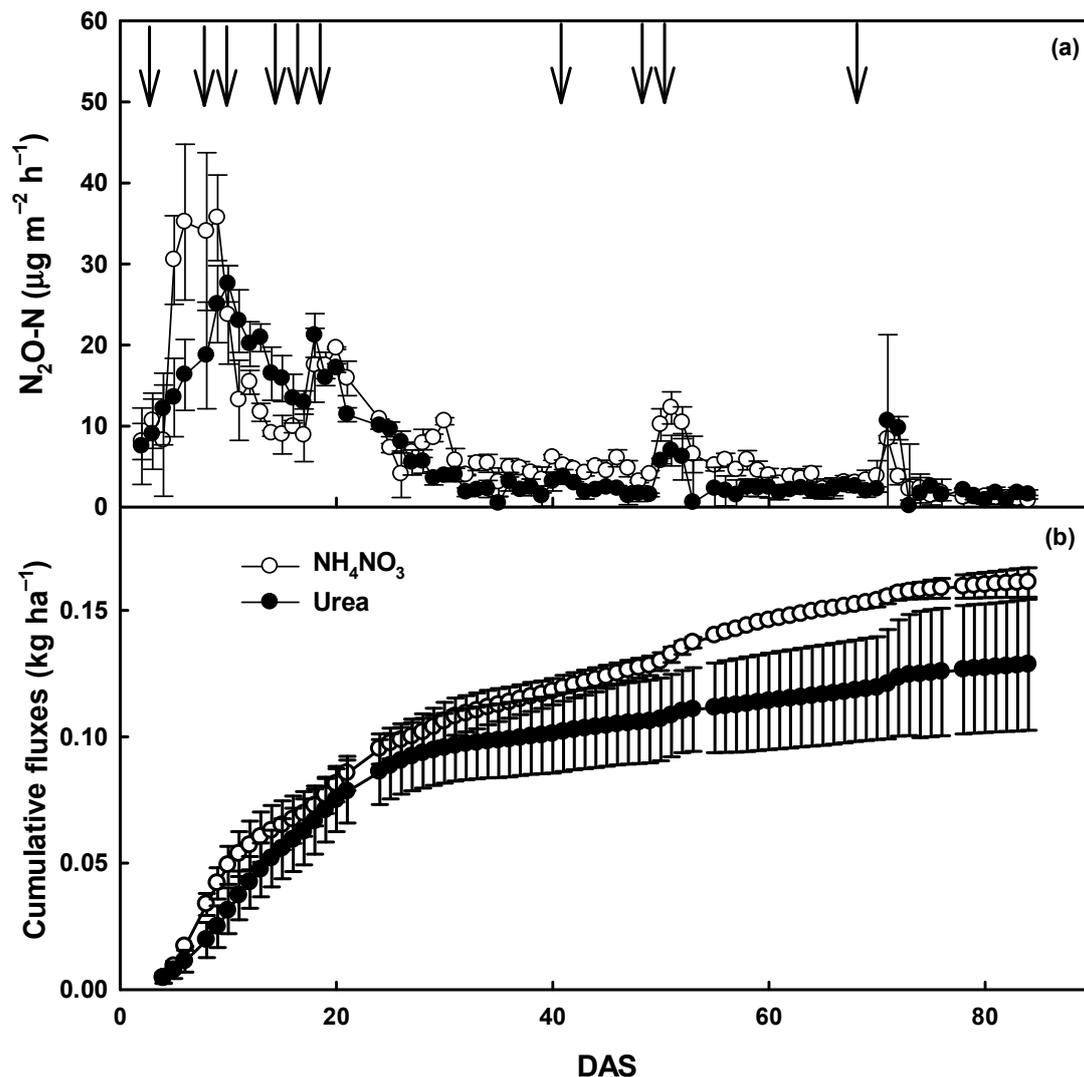


Figure 5. (a) Soil Nitrous oxide (N₂O-N) fluxes and (b) cumulative nitrous oxide (N₂O-N) from plots fertilized by ammonium nitrate (open circles) and urea (filled circles). The arrows denote irrigation events. Data are means ($n = 4$) \pm SE.

4. Discussion

Nitrogen (N) is often the most limiting factor in crop production and the application of nitrogen fertilizers to increasing rates results in higher biomass and seed yields with improved nutritional quality [4]. The use of fertilizers characterized by a slow N release should be advantageous for plants that should efficiently use the fertilizer and, in turn, to enhance seed yield and quality. In our experiment, we used urea as a slow N release fertilizer, and we observed its positive influence on hemp cultivation. In fact, urea fertilizer enhanced the plant biomass yield and reduced the N₂O emission from soil, but it had little influence on the quality of hemp seeds. Results reported here suggest that, at least in our experiment, the nitrogen fertilizer type seems to have little effect on the seed quality of Wojko hemp cultivar. Marked differences in seeds' composition were observed between CTR seeds and seeds obtained from plants grown under our experimental conditions, likely suggesting some influence of the weather conditions of cultivation site and/or of the agronomic management of crop on seed quality.

The results obtained in this study partially support our original hypothesis. Previous studies report an influence of nitrogen source not only on yield but also on quality of different crops [26–28]. In our study, fertilization with urea improved biomass yield. Previous findings reported an improvement on

plant growth [29,30] and enhanced N utilization [30] in response to urea fertilization as a consequence of augmented nitrogen use efficiency in response to the supply of urea fertilizer. This likely occurs in response to a higher nitrate content in the soil solution [31] that has been seen to have a positive effect on urea uptake [32].

The improved NUE in plants fertilized with urea was likely the consequence of the enhanced N uptake by plants due to the combined presence of both urea and nitrate in the soil that enhanced plant growth and the relative use of each N-source, as compared to nitrate or to urea when provided alone [32]. As a consequence, the nitrogen availability for microbes' transformations (mainly the nitrification) was likely limited, thereby reducing the N₂O production and emission in plots fertilized with urea, even if a loss of NH₃ following urea hydrolysis has to be hypothesized, likely reducing the net amount of N remaining in the soil as a potential source of N₂O.

Our results agree with most studies that report lower N₂O emission from soil fertilized with urea, while the NH₃ loss might partially explain why urea determines a lower EF value compared to ammonium nitrate. The lower average EF value for urea indicates a lower fraction of N applied lost as N₂O, a powerful greenhouse gas destroying stratospheric ozone and having a global warming potential 298 times greater than CO₂ on a 100-year time horizon [24]. We speculate that the lower EF for urea is mainly attributable to the improved use efficiency of nitrogen when plants are grown with urea in the presence of nitrate, the latter being a form of nitrogen present in the soil and deriving from organic matter degradation. Emission intensities provide information for reducing the relative impact of intensive agriculture on climate [33] since lower intensities denote more climate-friendly crops or management.

Seed quality is of paramount importance to agriculture and food security, while on the other hand, climate conditions or crop management affect seed quality. The results obtained in this study seem to indicate such a role of environmental conditions on macronutrient composition of hemp seeds, as suggested by the oleic/linoleic ratio (0.19, 0.24, and 0.23 respectively, in control, NH₄NO₃, and urea seeds). This ratio is strongly affected by temperatures during seed development [6], and the heterogeneity of the ratio found in this study between seeds from plants cultivated in Poland (CTR seeds) and seeds from plants cultivated in Italy under our experimental conditions (NH₄NO₃ and urea seeds) might suggest that oil quality obtained from seeds of the same cultivar might be different if specimens are cultivated in environments with different weather conditions, even if it is not possible to exclude such an influence of the agronomic management. However, under our experimental conditions, a small but significant difference between NH₄NO₃ and urea seeds was also found, implying some influence of nitrogen source on seed quality.

The average content of SFA (14–15%) and PUFA (68–69%) found in seeds deriving from NH₄NO₃ and urea treatments was respectively higher and lower than that reported by Baldini et al. [6] and Senila et al. [34]. Moreover, our results were higher than those reported by Orsavova et al. [35] for other hemp cultivars, also indicating an influence of cultivars on seed composition. The greater SFA content found in this study was due to a higher palmitic acid concentration, while stearic acid content showed comparable values to those reported elsewhere [6,34]. On the contrary, the lower PUFA content compared to values reported in Baldini et al. [6] was due to a lower concentration in linoleic acid and α -linoleic acid, whereas in Orsavova et al. [35], a lower α -linoleic acid content compared to our findings was reported. Omega-3 PUFA protects against cardiovascular diseases, and might be beneficial in rheumatoid arthritis, inflammatory bowel diseases, childhood learning and behavior, and adult psychiatric and neurodegenerative illnesses [36]. Previous studies show that fatty acids might act as antioxidants [37–39]. In particular, PUFAs such as linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (LNA, 18:3n-3), and MUFAs such as oleic acid (OA, 18:1n-9) limit the formation of reactive oxygen species (ROS), lowering the risk of ROS-induced diseases and enhancing the immune system. In the present study, even if the fatty acids composition is not influenced by nitrogen source, the ω -3/ ω -6 ratio in seed from plants grown with urea or ammonium nitrate was 0.31, within the ideal

range from a nutritional standpoint, being considered as the optimal proportion for human metabolism and health [40].

5. Conclusions

The findings reported here suggest that, at least in our experiment, the nitrogen source seems to have little influence on seed chemical composition, even if the ω -3/ ω -6 ratio in seeds from both fertilizers was within the optimal range from a nutritional standpoint. Urea fertilizer is more climate-friendly than ammonium nitrate fertilizer in terms of N₂O emission. However, to build on the findings discussed in this work, further research is necessary to have a better understanding of the influence of nitrogen source when compared over multiple years, soil types, varieties, and agro-climatic locations. On the other hand, other authors, not for hemp, who have worked on a single year, a single cultivar, a single soil type, and a single irrigation system, conclude that their results can be used to support management decisions for improving the production system [28]. Therefore, we believe that even if our results need further investigations, they might give guidelines to the farmers that are called to quickly implement agronomic practices modifications with an aim to mitigate the environmental impacts caused by agriculture.

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