



Nitrogen fertilization to optimize the greenhouse gas balance of hemp crops grown for biomass

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Abstract

Nitrogen trials were carried out on hemp crops grown in Ireland over a 3 year period to identify nitrogen fertilization strategies which optimize the greenhouse gas (GHG) and energy balances of hemp crops grown for biomass. Nitrogen rates up to 150 kg N ha⁻¹ were used in the study. Yield increased with nitrogen rate up to 120 kg N ha⁻¹ for early (Ferimon), mid (Felina 32) and late maturing (Futura 75) varieties. Variety had a significant effect on yield with yields increasing with maturation date. In 2 years of the study, certain application rates of nitrogen were applied either at sowing, at emergence, after emergence or split between these dates to determine if nitrogen rates could be reduced by delaying or splitting the applications. The application of nitrogen at times later than sowing or in splits during the early part of the growing season had no significant effect on biomass yield compared with the practice of applying nitrogen at the time of sowing. Late applications of nitrogen reduced leaf chlorophyll content and height early in the growing season. Later in the growing season, there was no difference in height between treatments although the highest concentrations of chlorophyll were found in the leaves of the late application treatment. Nitrogen rate and the timing of nitrogen application had no effect on plant density. Biomass yield, net energy and net GHG mitigation increased up to an application rate of 120 kg N ha⁻¹, this result was independent of soil type or soil nitrogen level. Net GHG and energy balance of hemp crops grown for biomass are optimized if late maturing varieties are used for biomass production and a nitrogen rate of 120 kg ha⁻¹ is applied at sowing.

Keywords: greenhouses gases, Hemp, leaf area index (LAI), nitrogen, rate, timing

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Introduction

Increasing oil prices, growing dependence on imported fossil energy together with growing evidence of the effect of GHG emissions on climate change (Solomon, 2007) are forcing countries to consider renewable forms of energy including bioenergy. Bioenergy, uniquely among renewable energy sources, can be used to provide energy either as heat, electricity or as a transport fuel. However, given the shortage of biomass from forestry production, energy crops are likely to play a major part in future production of bioenergy (Clifton-Brown *et al.*, 2007). Perennial energy crops such as willow and Miscanthus are expected to remain viable for up to 20 years (Bullard & Metcalf, 2001; Dawson, 2007) and are examples of good energy crops offering high biomass yields for low inputs while their perennial nature avoids soil disturbance. An alternative which is generating interest is the use of hemp as an energy crop. It has already been demonstrated that hemp is a suitable feedstock for anaerobic digestion (Kreuger *et al.*, 2011) as well as for combustion (Rice, 2008; Prade *et al.*, 2011).

Hemp can produce high biomass yields for low inputs (Meijer *et al.*, 1995; Crowley, 2001; Prade *et al.*, 2011), but is environmentally benign compared with other annual crops (Van der Werf, 2004). Hemp is an excellent break crop as its extensive root system improves soil structure. Subsequent crops have less weed pressure, and yield increases of 10%–20% have been demonstrated in winter wheat crops grown after hemp (Bosca & Karus, 1997). In addition, the fact that hemp is an annual crop offers farmers the opportunity to investigate the energy market without committing their land for up to 20 years if suitable markets exist.

Apart from diesel and seed, the only input required for the cultivation of hemp is fertilizer, as the crop is typically grown without inputs of herbicide, insecticide and fungicide (Crowley, 2001). Previous experience has led to the conclusion that fungicides are not beneficial and are not recommended (Van der Werf *et al.*, 1995a; ITC, 2007). The most important nutrients required for all crops are nitrogen, phosphorus and potassium. However, the most costly of these nutrients, in both economic and environmental terms, is nitrogen and crops are generally more responsive to nitrogen than to phosphorus or potassium (Hay & Walker, 1989). The effect of applied nitrogen on hemp yield has already been researched in a

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number of studies. The most extensive investigation to date was conducted by Struik *et al.* (2000) who found a gradual increase in stem dry matter over a range of 100 kg N ha⁻¹–220 kg N ha⁻¹ (soil N plus applied N) in Northern Europe. In contrast, there was a limited response to nitrogen in the nitrogen rich soils of Northern Italy (Struik *et al.*, 2000). Van der Werf *et al.* (1995b) found that yield was greater at a soil nitrogen level of 200 kg N ha⁻¹ compared with a soil nitrogen level of 80 kg N ha⁻¹ and concluded that the 80 kg N ha⁻¹ treatment was deficient in nitrogen. Amaducci *et al.* (2002) found that nitrogen fertilization increased stem production although Prade *et al.* (2011) reported no response to applied nitrogen. Most of the previous studies used a limited range of nitrogen levels applied at one time (sowing) and often at the higher densities used for fibre hemp production rather than at the lower densities used for biomass production (Crowley, 2001; Amaducci *et al.*, 2002). The objective of this study was to optimize the benefits of hemp as an energy crop by identifying the nitrogen fertilization strategies which maximize the GHG and energy balances of the crop.

Materials and methods

Nitrogen response trials were carried out on hemp crops grown in Ireland in 2008, 2009 and 2010. All experiments were designed as a randomized complete block with four replications. Certified seed was obtained from Cooperative Centrale des Producteurs de Semences de Chanvre, Le Mans, France and all crops were sown at a seeding rate of 30 kg ha⁻¹; this rate was previously found to give the highest biomass yields (Crowley, 2001). Trial areas were fertilized with phosphorus, sulphur and potassium prior to sowing (35 kg P ha⁻¹, 30 kg S ha⁻¹ and 150 kg K ha⁻¹) to ensure that supplies of all other major nutrients other than nitrogen were nonlimiting. Plots (2.2 m × 10 m) were sown with a Wintersteiger seed drill (Wintersteiger AG, 4910 Ried, Johann-Michael-Dimmelstrasse 9, Austria) at a row width of 15 cm and at a sowing depth of approximately 1 cm and rolled with a cambridge roller afterwards. Nitrogen treatments (calcium ammonium nitrate) were applied with a calibrated seed drill with coulters removed. No fungicides, insecticides or herbicides were applied during the study. The number of stems per plot was counted in each year of the study towards the end of each growing season. In addition, plant height was measured each year towards the end of the growing season and at the start of the growing season in 2009 and 2010 by measuring height on five plants selected at random from each plot and averaging five measurements from each plot. Similarly, leaf area index (LAI) was measured by making measurements at five locations selected at random in each plot and averaging those five measurements. LAI was measured using a Sunscan Canopy Analysis System (Delta-T Devices, 128 Low Road, Cambridge, CB25 0EJ, United Kingdom). Leaf chlorophyll concentration was measured on two occasions during 2009 using a chlorophyll meter (Minolta,

SPAD-502, Konica-Minolta, Tokyo, Japan), five measurements were taken at random in each plot on the middle leaf of the newest fully unfolded trifoliate leaf. Biomass was harvested at a cutting height of 5 cm. Harvesting in 2008 was accomplished by cutting a 1 m² quadrat from each plot. In 2009 and 2010, an area of 1.25 m × 2.2 m was harvested from the centre of each plot using an Agria reciprocating bar mower (Agria-Werke GmbH, Dittelbronner Str 42, D-74219, Mockmuhl, Germany). At harvest, the harvested material was weighed after which a sample of five stems was taken and separated into stem, leaf and flower. The fresh and dry weights of each of the plant components was determined before and after the material had been dried to constant weight at 90 °C. Meteorological information (precipitation, temperature and solar radiation) for the Oak Park and Knockbeg sites were taken from an automatic synoptic weather station at Oak Park as these two sites are located within a short distance of each other. Meteorological information (precipitation and temperature) for the site at Edenderry was obtained from a climatological weather station located on the farm at which measurements of rainfall and temperature were taken daily at 0900 GMT.

Hemp experiments 2008

Hemp trials were conducted at three sites during 2008: Oak Park, Knockbeg and Tong's Farm near Edenderry, Co Offaly. The sites at Oak Park and Knockbeg are situated within 5 km of each other, but are representative of two contrasting soil types, (Oak Park-eutric cambisol) and (Knockbeg-haplic luv-isol). Both sites are located on farms which are used for crop production and hemp was sown following spring barley in Oak Park and following winter wheat in Knockbeg. In contrast, the site near Edenderry was a former grassland site on a soil with a high peat content (calic gleysol). Each trial had variety and nitrogen levels as factors. An early maturing variety (Ferimon 12), a medium maturing variety (Felina 32) and a late maturing variety (Futura 75) were used in the trial. Five nitrogen levels were used 0, 70, 98, 119 and 150 kg N ha⁻¹. The sites at Oak Park and Knockbeg were sown on 11th April, nitrogen was applied to both sites on 15th April and both sites were harvested over 3rd – 4th September 2008. The site at Edenderry was sown on 16th April, nitrogen was applied on 28th April and the site was harvested on 11th September.

Hemp experiments 2009

One variety was used in 2009 (Futura 75). Trials were sown on two sites, Oak Park and Knockbeg, on 22nd April 2009 at a seeding rate of 30 kg ha⁻¹. The site at Oak Park had been left fallow the previous year following a spring barley crop while the site in Knockbeg had a spring barley crop the previous year. Levels of total soil nitrogen, soil organic matter and soil carbon were measured at both sites prior to sowing. Nitrogen treatments consisted of 0 kg N ha⁻¹, 60 kg N ha⁻¹ and 150 kg N ha⁻¹ applied at sowing, 90 kg N ha⁻¹, 120 kg N ha⁻¹ applied either at sowing (growth stage 0000), sowing plus 3 weeks (20 days, growth stage 1006), sowing plus 6 weeks (44 days, growth stage 1010) or split equally between sowing,

sowing plus 3 weeks and sowing plus 6 weeks. Growth stages were taken from Mediavilla *et al.* (1998). All trials were harvested on 3rd September.

Hemp experiments 2010

Hemp (Futura 75) was sown at two sites, Oakpark and Knockbeg on 14th April 2010 at a seeding rate of 30 kg ha⁻¹. The site in Oak Park had a winter oats crops the previous year while the site in Knockbeg was fallow the previous year following a spring barley crop. Levels of total soil nitrogen, soil organic matter and soil carbon were measured at both sites prior to sowing. Nitrogen treatments consisted of 0 kg N ha⁻¹ and 60 kg N ha⁻¹ applied at sowing, 90 kg N ha⁻¹, 120 kg N ha⁻¹ and 150 kg N ha⁻¹ applied either at sowing (growth stage 0000), sowing plus 2 weeks (14 days, growth stage 1004), sowing plus 4 weeks (29 days, growth stage 1008) or split equally between sowing, sowing plus 2 weeks and sowing plus 4 weeks. All trials were harvested on 9th September.

Energy Analysis and Life Cycle Assessment (LCA)

Net energy analysis and LCAs were conducted for each of the treatments where nitrogen fertilizer was applied at sowing in 2009 and 2010. The analysis included both the Oak Park and the Knockbeg sites. The functional unit used for both the LCA and the energy analysis was 1 hectare over a time period of 1 year. The systems boundary extended from sowing to harvesting and included energy and GHG emissions from fertilizer manufacture.

Agronomic operations consisted of ploughing, tilling, sowing, fertilization, rolling and harvesting. Crowley (2001) established that hemp could be grown in Ireland without the aid of agrochemicals and that low seeding rate (30 kg ha⁻¹) could be used for biomass production where fibre quality is not important. It was assumed that fertilizer would be applied at sowing using either a combine drill or a single pass system and that the crop would be mown before being swathed and baled during harvest.

In the first instance, it was necessary to construct average farm models representing each system, following the example of Casey & Holden (2004) and based on Styles & Jones (2007). All relevant inputs to the system and induced processes (e.g. soil N₂O emissions) were then considered in a life cycle inventory up to the point of harvest. All major inputs and sinks of the major greenhouse gases (GHGs), CO₂, CH₄ and N₂O were considered. Soil N₂O emissions were calculated in two ways. The first way was according to IPCC methodology by multiplication of the amounts of N added to the soil by a constant emission factor of 1% of applied nitrogen (de Klein *et al.*, 2006).

It has also been found that soil N₂O emissions may increase exponentially with N additions from chemical fertilizer (McSwiney & Robertson, 2005; Cardenas *et al.*, 2010) and also total N application from slurry, fertilizer and excretion (Rafique *et al.*, 2011). This pattern may be caused by a reduction in the capacity of the crop to take up N at high application rates (McSwiney & Robertson, 2005). Therefore, we estimated an alternative set of N₂O emissions by an exponential function relating N inputs to N₂O emissions in Ireland (Rafique *et al.*, 2011). Inventory mass

balances were summed and converted into a final Global Warming Potential expressed as kg CO₂ eq considered over a 100 year timescale, according to IPCC (2006) guidelines (CO₂ = 1, LCH₄ = 23, N₂O = 296). LCA outputs were calculated and expressed as kg CO₂ eq per hectare of land and per year.

Energy use was divided into two categories of uses, activities which used primarily diesel and those activities which used primarily electricity. A lower heating value of 35.9 MJ kg⁻¹ for oil was used (Dalgaard *et al.*, 2001) and GHG emissions were calculated according to Flessa *et al.* (2002) and included indirect emissions. Lubrication oil emissions were calculated as 5% of farm machinery diesel emissions (Dalgaard *et al.*, 2001). Greenhouse gas production from electricity usage was calculated using the 2004 GHG intensity of delivered electricity (0.173 kg CO₂ eq MJ_e⁻¹) after conversion of primary energy requirement values to delivered electricity based on an efficiency factor of 0.406 (Howley *et al.*, 2006). Indirect emissions associated with agricultural machinery production and maintenance were assumed to be proportional to fuel consumption following the method of Dalgaard *et al.* (2001). Fertilizer manufacturing, packaging and transport energy intensities of 79.6, 34.5 and 10.5 MJ kg⁻¹ for N, P, K and S were used to which were added manufacturing N₂O emissions of 9.63 g kg⁻¹ N (Elsayed *et al.*, 2003). Combined manufacturing and calcification emissions quoted by Elsayed *et al.* (2003) were divided into manufacturing and soil emissions based on an energy requirement of 6.43 MJ kg⁻¹.

Gross energy produced by the crop was calculated using a gross calorific value of 18.5 MJ/kg (Rice, 2008). The calculation of gross GHG mitigation assumed that biomass from hemp would replace oil and used a figure of 0.44 tonnes of carbon mitigated per tonne of dry matter (Cannell, 2003). Energy used during the life cycle was subtracted from gross energy to calculate net energy produced while GHG emitted during the life cycle were subtracted from gross GHG mitigation from each treatment to calculate net GHG mitigation.

Statistics

The results from each year were analysed separately by analysis of variance using PROC GLM procedure in SAS (SAS/STAT® v.9.2. 2009. SAS Institute Inc. Cary, NC). In 2008, site, variety and nitrogen level were the main factors.

In 2009 and 2010, only one variety was used, but nitrogen application timing was introduced as an additional factor. In each of these years, the treatments for which nitrogen timing was varied were analysed separately in an analysis in which site, timing and nitrogen level were the main factors. Treatments common to both years (0, 60, 90, 120, 150 kg N ha⁻¹ applied at sowing) were analysed together in an analysis in which year, site and nitrogen level were the main factors.

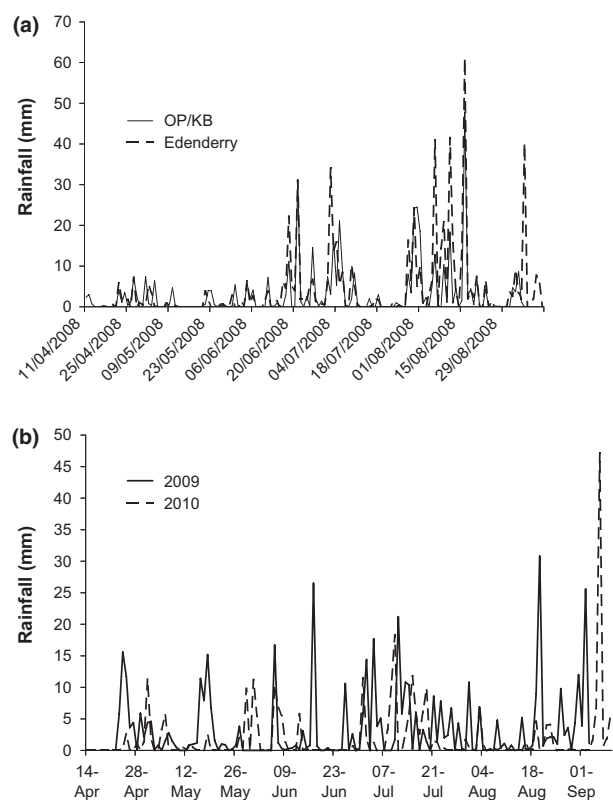
Results

Meteorological Conditions

Meteorological parameters recorded during the course of the trial are shown in Table 1 and in Fig. 1. The site

Table 1 Meteorological Parameters recorded at the sites used for the experiments over the period of the hemp growing season (date of sowing to date of harvest)

	Precipitation (mm)	Average Temp(°C)	Solar Radiation (J/cm ⁻²)
2008			
Oak Park/Knockbeg	453.4	12.6	1388.7
Edenderry	643.2	12.6	
2009			
Oak Park/Knockbeg	467.7	13.7	1487.7
2010			
Oak Park/Knockbeg	280.3	13.4	1518.4

**Fig. 1** (a) Rainfall at all three sites during 2008, Edenderry and Oak Park/Knockbeg. (b) rainfall at the Oak Park and Knockbeg sites during 2009 and 2010.

at Edenderry had higher rainfall compared with the Oak Park and Knockbeg sites in 2008 although ambient temperatures were similar. Rainfall was relatively low until the end of June after which rainfall increased at all sites although rainfall was heaviest at the Edenderry site (Fig. 1a). Ambient temperatures and levels of solar radiation in 2009 and 2010 were similar at the Oak Park and Knockbeg sites. Rainfall levels, however, differed

between 2009 and 2010. Rainfall levels in 2010 were substantially lower than those in 2009 (Fig. 1b). 111.7 mm of rainfall fell in the first 30 days after sowing in 2009 whereas 30.4 mm of rainfall fell in the first 30 days after sowing in 2010.

2008 experiments

There were significant differences in total yield, stem yield and leaf yield between the three sites used in 2008 (Table 2), the highest yields were obtained from the Oak Park site where the yields were significantly higher than both the Knockbeg and the Edenderry sites. Nitrogen rate had a significant effect ($P < 0.0001$) on total yield and on the yield of all plant components (stem, leaf and flower). Yields increased with added nitrogen, but had levelled off at an application rate of 120 kg ha⁻¹, there was no significant difference between the 120 kg ha⁻¹ and the 150 kg ha⁻¹ treatments. Yield increased with increasing varietal maturation date, variety had a significant effect on total yield ($P < 0.01$), stem yield ($P < 0.0001$) and flower yield ($P < 0.0001$), but not leaf yield. Total yield, stem yield and flower yield of Futura 75 were significantly higher than those of both Ferimon and Felina 32. There was a significant site by variety interaction for total yield ($P < 0.05$) and leaf yield ($P < 0.0001$), but not for the other yield components. There were significant interactions between site and nitrogen for total yield ($P < 0.01$) and stem yield ($P < 0.01$). This was because yield reached a plateau at an application rate of 120 kg N ha⁻¹ before levelling out at both the Oak Park and Knockbeg sites whereas yield reached a maximum at 120 kg N ha⁻¹ before declining at the Edenderry site. Hence, the shape of the nitrogen response curves differed. There were no significant variety by nitrogen interactions with the exception of flower yield ($P < 0.05$) and there was no significant site by variety by nitrogen interaction.

Plant density did not differ significantly between site or nitrogen treatments although there were significant differences in plant density between the varieties used in these trials ($P < 0.001$). LAI differed significantly between sites ($P < 0.0001$), differences in LAI between sites corresponded to yield differences between sites. LAI increased with nitrogen rate ($P < 0.0001$) although there were no significant differences between the LAI of the 120 kg N ha⁻¹ and the 150 kg N ha⁻¹ treatments. The interaction between site and nitrogen rate was statistically significant for LAI ($P < 0.0001$). Plant height differed significantly between sites ($P < 0.001$); plant height at the Oak Park site was significantly greater than plant height at both the Knockbeg and Edenderry sites. Similar to the LAI parameter, differences in height between sites corresponded to differences in site yield.

Table 2 Results obtained from nitrogen trials carried out with three varieties of hemp grown at three sites in 2008. All yield data are in tonnes of dry matter per hectare. Means followed by the same letter are not significantly different

	Total Yield	Stem Yield	Leaf Yield	Flower Yield	Plants/m ² 23/7	Leaf Area Index (LAI) 15/7	Height (cm) 15/7
Site							
Oak Park	12.4 ± 3.5 A	10.4 ± 3.1 A	1.0 ± 0.5 A	1.0 ± 0.6 A	146.8 ± 23.7 A	8.2 ± 1.8 A	176.7 ± 22.7 A
Knockbeg	11.2 ± 3.8 B	8.8 ± 3.1 B	1.4 ± 0.5 B	1.0 ± 0.4 A	139.9 ± 21.6 A	7.1 ± 1.7 B	169.7 ± 26.2 B
Edenderry	11.0 ± 2.8 B	9.0 ± 2.3 B	1.1 ± 0.4 A	1.0 ± 0.4 A	141.8 ± 25.8 A	6.6 ± 1.0 C	166.6 ± 14.8 B
Variety							
Ferimon	11.1 ± 3.5 A	8.8 ± 2.8 A	1.1 ± 0.4 A	1.2 ± 0.4 A	142.3 ± 24.9 AB	7.1 ± 1.7 A	166.0 ± 21.7 A
Felina 32	11.2 ± 3.3 A	8.9 ± 2.7 A	1.2 ± 0.4 A	1.1 ± 0.4 A	151.6 ± 23.4 A	7.3 ± 1.7 A	170.0 ± 21.3 A
Futura 75	12.2 ± 3.4 B	10.5 ± 3.0 B	1.1 ± 0.6 A	0.7 ± 0.4 B	134.7 ± 20.2 B	7.4 ± 1.5 A	177.0 ± 22.1 B
Nitrogen							
0 kg N ha ⁻¹	6.4 ± 1.8 A	5.1 ± 1.6 A	0.6 ± 0.2 A	0.6 ± 0.3 A	149.1 ± 21.6 A	5.0 ± 0.8 A	140.6 ± 20.0 A
70 kg N ha ⁻¹	11.1 ± 2.0 B	9.0 ± 1.7 B	1.1 ± 0.5 B	1.0 ± 0.4 B	144.2 ± 25.9 A	7.0 ± 1.0 B	172.5 ± 14.0 B
98 kg N ha ⁻¹	12.4 ± 1.9 C	10.2 ± 1.7 B	1.2 ± 0.4B C	1.1 ± 0.4 BC	144.2 ± 23.6 A	7.7 ± 1.1 C	177.0 ± 13.9 BC
120 kg N ha ⁻¹	13.8 ± 2.5 D	11.2 ± 2.3 C	1.4 ± 0.5 C	1.2 ± 0.5 C	139.8 ± 24.7 A	8.2 ± 1.2 D	182.1 ± 15.4 C
150 kg N ha ⁻¹	14.0 ± 1.7 D	11.3 ± 1.7 C	1.4 ± 0.5 C	1.3 ± 0.5 C	137.0 ± 22.5 A	8.5 ± 1.3 D	182.9 ± 14.4 C
Statistics (<i>P</i> values)							
Site	0.0001	<0.0001	<0.0001	0.5353	0.2397	<0.0001	0.0003
Variety	0.0013	<0.0001	0.1101	<0.0001	0.0004	0.0624	<0.0001
Nitrogen	<0.0001	<0.0001	<0.0001	<0.0001	0.2135	<0.0001	<0.0001
Site × Variety	0.0429	0.2246	<0.0001	0.9661	0.1347	0.0841	0.3163
Site × Nitrogen	0.0079	0.0028	0.1071	0.1947	0.3451	<0.0001	<0.0001
Variety × Nitrogen	0.8458	0.3535	0.7608	0.0454	0.8500	0.8036	0.8887
Site × Variety × Nitrogen	0.9490	0.9675	0.1465	0.3602	0.5860	0.7219	1.000

Plant height increased with increasing varietal maturation date ($P < 0.0001$) and with increasing nitrogen supply ($P < 0.0001$), but there was a significant interaction between site and nitrogen level ($P < 0.0001$). There were no significant interactions between site and variety, between variety and nitrogen and between site, variety and nitrogen for the LAI and height parameters.

2009 Timing of Nitrogen Application Treatments

The timing of nitrogen application was varied for two treatments in 2009, 90 kg N ha⁻¹ and 120 kg N ha⁻¹. There were four timing treatments for each of these two levels of nitrogen application and these treatments were analysed separately from the other treatments in the trials, results are shown in Table 3. Total yield as well as stem yield and leaf yield differed significantly between the two sites, yields at the Knockbeg site were significantly higher than the Oak Park site primarily due to a significant reduction in plant density at the Oak Park site thought to be primarily attributable to bird damage. However, although establishment was reduced this effect was uniform across the experimental area. There

were no significant differences in total yield, stem yield and flower yield between the different timing treatments. Flower yield was not significantly affected by site, timing or nitrogen level. There were no significant interactions between site and timing, site and nitrogen, timing and nitrogen or between site, nitrogen and timing for any of the yield components. Timing of nitrogen application or nitrogen rate had no significant effect on plant density.

The timing of the nitrogen treatment had a significant effect on leaf chlorophyll content measured on 11th June ($P < 0.0001$). Leaf chlorophyll concentration on this date was highest in the treatment which received nitrogen at sowing and decreased as the date of nitrogen application was delayed. The timing of the nitrogen treatments also had a significant effect on leaf chlorophyll concentration measured on 23rd July although on this date the ordering of the timing treatments was reversed as the chlorophyll concentration in the leaves of the sowing + 44 treatment was significantly greater than the chlorophyll concentration in all the other timing treatments. Nitrogen timing had a significant ($P < 0.0001$) effect on plant height measured on 12th June. Plant height

Table 3 Results obtained in 2009 from the variety Futura 75 grown at two sites and fertilized with two nitrogen application rates applied either at sowing, 20 days after sowing, 44 days after sowing or split between these three dates. All yield data are in tonnes of dry matter per hectare. Means followed by the same letter are not significantly different

Site	Total Yield	Stem Yield	Leaf Yield	Flower Yield	Plants/m ² 27/7	Chlorophyll (SPAD units)		Height (cm) 12/6	Height (cm) 28/7	Leaf Area Index (LAI)	
						11/06	23/07			23/07	11/8
Oak Park	8.9 ± 2.7 A	7.1 ± 2.3 A	1.2 ± 0.3 A	0.5 ± 0.2 A	37.8 ± 11.7 A	32.9 ± 2.7 A	33.7 ± 2.2 A	33.0 ± 10.1 A	217.0 ± 24.8 A	3.0 ± 0.8 A	
Knockbeg	11.5 ± 2.0 B	9.5 ± 1.7 B	1.4 ± 0.3 B	0.6 ± 0.2 A	97.0 ± 17.5 B	32.0 ± 5.4 A	32.2 ± 1.7 B	46.8 ± 10.3 B	223.4 ± 10.6 A	4.1 ± 1.1 B	
Timing											
Sowing	11.1 ± 2.9 A	9.2 ± 2.5 A	1.3 ± 0.4 AB	0.6 ± 0.2 A	68.4 ± 31.3 A	35.2 ± 2.3 A	32.4 ± 1.9 A	46.1 ± 12.6 A	224.5 ± 17.7 A	3.5 ± 1.1 AB	
Sowing + 20	9.3 ± 2.8 A	7.6 ± 2.5 A	1.2 ± 0.3 B	0.5 ± 0.1 A	63.2 ± 29.7 A	33.0 ± 2.7 B	32.1 ± 1.6 A	41.9 ± 9.7 A	212.9 ± 16.7 A	3.2 ± 1.1 B	
Sowing + 44	10.8 ± 2.2 A	8.7 ± 1.8 A	1.5 ± 0.4 A	0.6 ± 0.2 A	68.4 ± 39.4 A	27.4 ± 3.7 C	34.8 ± 2.0 B	26.8 ± 4.7 B	225.1 ± 18.8 A	4.3 ± 1.0 A	
Split	9.5 ± 2.8 A	7.8 ± 2.4 A	1.2 ± 0.3 B	0.5 ± 0.2 A	69.7 ± 34.9 A	34.1 ± 3.2 AB	32.5 ± 1.9 A	44.9 ± 10.1 A	218.4 ± 22.3 A	3.2 ± 0.9 B	
Nitrogen											
90 kg N ha ⁻¹	9.1 ± 2.6 A	7.4 ± 2.2 A	1.1 ± 0.3 A	0.5 ± 0.2 A	66.3 ± 36.3 A	32.5 ± 4.7 A	32.5 ± 1.7 A	39.8 ± 12.3 A	213.4 ± 20.8 A	3.2 ± 1.0 A	
120 kg N ha ⁻¹	11.2 ± 2.5 B	9.2 ± 2.1 B	1.5 ± 0.3 B	0.6 ± 0.2 A	68.6 ± 30.6 A	32.3 ± 3.8 A	33.5 ± 2.3 B	40.0 ± 12.4 A	227.0 ± 14.8 B	3.9 ± 1.0 B	
Statistics (<i>P</i> values)											
Site	<0.0001	<0.0001	0.0116	0.2187	<0.0001	0.1620	0.0004	<0.0001	0.1424	<0.0001	
Timing	0.0410	0.0490	0.0170	0.1206	0.6587	<0.0001	<0.0001	<0.0001	0.1569	0.0018	
Nitrogen	0.0002	0.0003	<0.0001	0.2007	0.5607	0.7395	0.0097	0.9087	0.0023	0.0014	
Site × Timing	0.6688	0.5158	0.8358	0.4624	0.4740	<0.0001	0.8160	0.1849	0.1340	0.7859	
Site × Nitrogen	0.4596	0.4529	0.1985	0.4894	0.0783	0.9500	0.0020	0.7932	0.2079	0.7930	
Timing × Nitrogen	0.6982	0.7061	0.3552	0.9885	0.9487	0.1797	0.8093	0.5048	0.6027	0.1350	
Site × Timing × Nitrogen	0.3602	0.3209	0.4376	0.9243	0.9656	0.7549	0.6830	0.4021	0.1780	0.8360	

decreased as nitrogen timing was delayed; plant height in the sowing + 44 treatment was significantly lower compared with the plant height in all other treatments. In contrast, timing had no significant effect on plant height measured towards the end of the growing season. Similarly, site had no significant effect on plant height on this date although height increased with nitrogen rate ($P < 0.01$). LAI measured towards the end of the growing season differed significantly between sites ($p < 0.0001$), differences in LAI between sites corresponded to yield differences between sites and increased with nitrogen level ($P < 0.01$). Timing of nitrogen application had a significant effect on LAI ($P < 0.01$), the effect of nitrogen timing on LAI mirrored the effect of timing on leaf yield. The highest leaf yield and LAI were obtained from the sowing + 44 timing treatment. There were no significant interactions between site and timing, between site and nitrogen, between timing and nitrogen and between site, variety and nitrogen for the LAI and height parameters.

2010 Timing of Nitrogen Application Treatments

The timing of nitrogen application was varied for three treatments in 2009, 90 kg N ha⁻¹, 120 kg N ha⁻¹ and 150 kg N ha⁻¹. There were four timing treatments for each of these three levels of nitrogen application and these were analysed separately from the other treatments in the trials, results are shown in Table 4. Total yield and stem yield were significantly higher at the Oak Park site compared with the Knockbeg site whereas leaf and flower yields were significantly higher ($P < 0.001$) at the Knockbeg site. The timing of nitrogen application did not have a significant effect on any of the yield parameters. There was no significant timing by nitrogen or site by timing by nitrogen interactions on any of the yield components with the exception of flower yield ($P < 0.05$). There was a significant difference in plant density between sites ($P < 0.0001$), but nitrogen rate and timing had no significant effect on plant density. Nitrogen timing had a significant effect ($P < 0.0001$) on plant height measured on 12th June, plant height decreased with delay in nitrogen application. In contrast, timing had no significant effect on height measured towards the end of the growing season. On both dates, nitrogen rate had a significant effect on plant height ($P < 0.01$). LAI differed significantly between sites ($P < 0.0001$) and reflected differences in yield between the two sites. However, nitrogen rate had no significant effect on LAI. There were no significant interactions between site, nitrogen rate and nitrogen timing for the LAI and height parameters with the exception of a statistically significant interaction ($P < 0.05$) between site and nitrogen rate for LAI.

2009/2010 Analysis of Common Treatments

Levels of total soil nitrogen, soil carbon and soil organic matter at both sites in both years of the study are shown in Table 5. The Oak Park site had higher levels of nitrogen, organic matter and carbon in both years of the study. In 2010, levels of total soil nitrogen were twice as high in the Oak Park site compared with the Knockbeg site. The results of the analysis of treatments common to both 2009 and 2010 is shown in Table 6. Stem yields were significantly ($P < 0.05$) higher in 2010 compared to 2009 and this was most probably attributable to higher plant numbers ($P < 0.0001$) in 2010. Yields did not differ significantly between sites, but applied nitrogen had a significant effect on total yield, stem yield, leaf yield and flower yield ($P < 0.0001$). Yields increased with added nitrogen, but had levelled off at an application rate of 120 kg ha⁻¹, there were no significant differences between the 120 kg N ha⁻¹ and the 150 kg N ha⁻¹ treatments across any of the yield components. There were no significant site by nitrogen interactions; the response to nitrogen fertilizer did not differ between sites in spite of the fact that there were large differences in soil nitrogen between the two sites. There was a significant year by site interaction for total yield and stem yield ($P < 0.0001$) and for flower yield ($P < 0.05$). Year had a significant impact on the response to nitrogen of leaf yield and flower yield ($p < 0.01$), but not stem yield and total yield. Net energy yield and net GHG mitigation were significantly higher in 2010 compared to 2009 ($P < 0.05$). Net energy increased with nitrogen rate ($P < 0.0001$) up to a nitrogen rate of 120 kg N ha⁻¹, but there were no significant differences in net energy or net GHG mitigation between the 120 kg N ha⁻¹ and the 150 kg N ha⁻¹ treatments. Net GHG mitigation showed the same trend with no significant difference between the 120 kg N ha⁻¹ and the 150 kg N ha⁻¹ treatments irrespective of whether soil N₂O emissions were calculated on the basis of a linear trend or an exponential trend. However, net GHG mitigation was lower when soil N₂O emissions were calculated on the basis of an exponential trend. There were significant differences ($P < 0.0001$) in plant numbers between sites and years, but nitrogen rate had no significant effect on plant numbers.

Discussion

In intensive agriculture, soil and tissue levels of phosphorus, potassium, calcium, magnesium, sulphur and trace elements can usually be adjusted to fall in the optimum range leaving nitrogen as the major nutrient factor determining crop yield (Hay & Walker, 1989). Thus, nitrogen plays a crucial role in the economics of crops,

Table 4 Results obtained in 2010 from the variety Futura 75 grown at two sites and fertilized with three nitrogen application rates applied either at sowing, 14 days after sowing, 29 days after sowing or split between these three dates. All yield data are in tonnes of dry matter per hectare. Means followed by the same letter are not significantly different

	Total Yield	Stem Yield	Leaf Yield	Flower Yield	Plants/m ² 9/9	Height (cm) 12/6	Height (cm) 16/8	Leaf Area Index (LAI) 16/8
Site								
Oak Park	13.5 ± 1.9 A	11.7 ± 1.8 A	0.9 ± 0.2 A	0.9 ± 0.3 A	178.2 ± 48.7 A	125.1 ± 7.8 A	236 ± 21.9 A	5.3 ± 0.8 A
Knockbeg	12.6 ± 2.4 B	10.5 ± 2.1 B	1.1 ± 0.2 B	1.0 ± 0.3 B	256.1 ± 59.4 B	89.3 ± 8.5 B	233.9 ± 25.0 A	4.3 ± 1.0 B
Timing								
Sowing	13.0 ± 1.9 A	11.0 ± 1.9 A	1.0 ± 0.2 A	1.0 ± 0.2 A	229.0 ± 72.4 A	111.5 ± 18.8 A	236.6 ± 19.4 A	4.9 ± 0.8 A
Sowing + 14	13.1 ± 2.6 A	11.2 ± 2.4 A	1.0 ± 0.2 A	0.9 ± 0.4 A	207.1 ± 56.9 A	108.2 ± 24.4 A	238.4 ± 30.0 A	4.9 ± 1.1 A
Sowing + 29	13.2 ± 1.8 A	11.2 ± 1.7 A	1.0 ± 0.2 A	0.9 ± 0.3 A	216.5 ± 50.9 A	100.5 ± 17.4 B	233.8 ± 23.8 A	4.7 ± 1.3 A
Split	13.1 ± 2.4 A	11.1 ± 2.2 A	1.1 ± 0.3 A	0.9 ± 0.3 A	215.8 ± 84.6 A	108.5 ± 16.8 A	232.2 ± 19.9 A	4.7 ± 0.8 A
Nitrogen								
90 kg N ha ⁻¹	11.9 ± 2.5 A	10.1 ± 2.4 A	0.9 ± 0.2 A	0.9 ± 0.2 A	202.3 ± 64.8 A	104.7 ± 19.6 A	225.1 ± 23.8 A	4.6 ± 1.2 A
120 kg N ha ⁻¹	13.2 ± 1.6 B	11.2 ± 1.6 B	1.0 ± 0.2 AB	0.9 ± 0.3 A	227.2 ± 62.5 A	106.3 ± 21.1 A	234.2 ± 23.6 AB	4.9 ± 1.0 A
150 kg N ha ⁻¹	14.1 ± 1.9 B	12.0 ± 1.6 B	1.1 ± 0.2 B	1.0 ± 0.3 A	220.3 ± 72.3 A	110.4 ± 18.4 B	245.9 ± 18.4 B	5.0 ± 0.8 A
Statistics (P values)								
Site	0.0188	0.0007	0.0007	0.0009	<0.0001	<0.0001	0.6208	<0.0001
Timing	0.9699	0.9301	0.8889	0.4009	0.5709	<0.0001	0.7368	0.8589
Nitrogen	<0.0001	<0.0001	0.0167	0.1964	0.1764	0.0035	0.0018	0.1892
Site × Timing	0.1070	0.0801	0.5414	0.0136	0.0257	0.0079	0.059	0.8076
Site × Nitrogen	0.0031	0.0043	0.8441	0.0017	0.7178	0.3164	0.1653	0.0175
Timing × Nitrogen	0.0832	0.1032	0.8051	0.0497	0.8679	0.7438	0.5285	0.8115
Site × Timing × Nitrogen	0.9648	0.9778	0.6610	0.0403	0.9978	0.7261	0.6039	0.6848

Table 5 Soil results from experiments conducted in 2009 and 2010

	Organic Matter (%)	Soil C (%)	Total soil N (%)
2009 Oak Park	5.6	2.7	0.3
2009 Knockbeg	4.4	2.4	0.2
2010 Oak Park	6.8	3.5	0.4
2010 Knockbeg	4.2	2.2	0.2

but also in the environmental balance of crops as GHG emissions during the manufacture and application of nitrogenous fertilizers are significant (Wood & Cowie, 2004; IPCC, 2006) while nitrogen leached from applications of nitrogenous fertilizer may contribute to pollution in groundwater and surface water. As nitrogen is typically the most important factor in determining crop yield, it is important not only to determine the optimum level of fertilization of a given crop but also to determine how the fertilizer should be distributed over time to provide maximum effect.

Nitrogen fertilization influences crops primarily through its effect on leaf size and longevity, increases in canopy size ultimately lead to increases in crop dry matter production as nitrogen fertilization does not normally influence photosynthetic rates (Hay & Walker, 1989). In this study, the application of nitrogen increased LAI as found previously by Van der Werf *et al.* (1995b) and biomass yield was directly related to both LAI and to plant height. Thus, higher assimilation rates in larger canopies produced taller stems. It is also possible that nitrogen fertilization resulted in wider stems in addition to taller stems as Van der Werf *et al.* (1995b) reported that nitrogen fertilization increased biomass yield by increasing stem diameter although they found no effect on plant height. Flowering date had a significant effect on yield as canopy duration was restricted in the early flowering varieties with the result that these varieties did not grow as tall as the late maturing variety, Futura 75. Thus, higher yields can be expected from late maturing varieties as a result of longer canopy duration. Plant density was unaffected by nitrogen rate in this study in contrast to previous studies which found an increase in plant mortality with applied nitrogen (Van der Werf *et al.*, 1995b; Struik *et al.*, 2000; Amaducci *et al.*, 2002). Decreases in plant density at higher nitrogen fertilization levels have been attributed to enhanced shading (Van der Werf *et al.*, 1995b) although Struik *et al.* (2000) did not find a significant effect of nitrogen fertilization on plant density at all sites and reported that overall, the effect of nitrogen application on plant density was small.

A wide range of responses to nitrogen have been reported for hemp. Prade *et al.* (2011) conducted experiments in Sweden on a humus rich soil and found no

yield response to applied nitrogen up to 200 kg N ha⁻¹. Similarly, Struik *et al.* (2000) reported on hemp fertilization experiments in Italy conducted on a soil which was reported to be very rich in nitrogen and found no response in 1 year and only a small response in a 2nd year. In contrast, Amaducci *et al.* (2002) reported that each kg of nitrogen increased stem yield by 20 Kg. Struik *et al.* (2000) found a stronger response to nitrogen in Northern Europe where there was a gradual increase in stem yield with applied nitrogen. Van der Werf *et al.* (1995b) also found a positive response to applied nitrogen while Iványi *et al.* (1997) found a positive response to applied nitrogen up to an application rate of 160 kg N ha⁻¹. The results of this study suggest that an application rate of 120 kg N ha⁻¹ is adequate for the range of soil types used in the study as yield response to nitrogen reached a maximum at 120 kg N ha⁻¹ on all of the soil types used in this study in spite of the fact that there were large differences in total soil nitrogen and soil organic matter between sites. This finding corresponds to advice provided in France (ITC, 2007) and to more general advice provided by Bosca & Karus (1997). Soils rich in nitrogen, however, such as organic soils may require lower applications of nitrogen as found by Struik *et al.* (2000) and Prade *et al.* (2011). In contrast, exhausted soils may require higher applications of nitrogen to obtain economic yields of hemp.

Higher yields are typically obtained when the application of nitrogen to a crop is split between different stages of crop growth. Gehl *et al.* (2005) examined the response of maize to nitrogen application at a number of sites and found that maximum grain yield at all sites was obtained when a split application of nitrogen was used. In this way, nitrogen is supplied according to the needs of the crop (Hay & Walker, 1989). For example, crops cannot absorb nitrogen until they have developed a sufficient root system and in any case there are often sufficient supplies of nitrogen in the seed/soil to support initial growth. Fertilizer applied at sowing is often not needed immediately and, in any case, cannot be taken up initially. Consequently, nitrogen applied at sowing can often be washed from the soil by rainfall and its economic value is lost to the farmer. Hemp maintains a large canopy into early August and, consequently, might be expected to benefit from later applications of nitrogen. However, our results suggest that there is no benefit either to nitrogen applications after sowing or to split applications of nitrogen during the growing season. This result was obtained in a dry year (2010) when only 33.5 mm of rainfall fell in the first 40 days after sowing as well as in a wet year when 118.4 mm of rainfall fell in the same period with a consequently greater risk of leaching. The converse would usually be expected as a result of some of the nitrogen applied at sowing being

Table 6 Results obtained from nitrogen response trials in 2009 and 2010 in which the variety Futura 75 was grown at two sites and fertilized with different application rates of nitrogen fertilizer applied at sowing. All yield data are in tonnes of dry matter per hectare. Means followed by the same letter are not significantly different

Site	Total Yield	Stem Yield	Leaf Yield	Flower Yield	Plants/m ²	Net Energy Yield (GJ/ha)	Net GHG mitigation (t CO ₂ eq/ha) IPCC N ₂ O method	Net GHG mitigation (t CO ₂ eq/ha) Rafique <i>et al.</i> , N ₂ O method
Oak Park	10.1 ± 4.0 A	8.4 ± 3.5 A	1.0 ± 0.4 A	0.7 ± 0.3 A	112.3 ± 82.3 A	138.5 ± 61.4 A	11.2 ± 5.1 A	10.7 ± 5.2 A
Knockbeg	10.0 ± 4.0 A	8.3 ± 3.4 A	1.0 ± 0.3 A	0.7 ± 0.3 A	176.6 ± 87.4 B	136.5 ± 58.7 A	11.0 ± 4.8 A	10.5 ± 4.9 A
Year								
2009	9.7 ± 4.1 A	7.9 ± 3.4 A	1.2 ± 0.5 A	0.6 ± 0.3 A	73.7 ± 39.3 A	130.1 ± 58.0 A	10.4 ± 4.9 A	10.0 ± 5.0 A
2010	10.4 ± 3.8 A	8.7 ± 3.4 B	0.9 ± 0.3 B	0.8 ± 0.3 B	215.2 ± 68.7 B	144.9 ± 59.7 B	11.7 ± 4.9 B	11.3 ± 5.0 B
Nitrogen								
0 kg N ha ⁻¹	5.0 ± 1.8 A	4.0 ± 1.5 A	0.6 ± 0.1 A	0.4 ± 0.3 A	137.4 ± 79.6 A	64.0 ± 27.7 A	5.3 ± 2.4 A	4.7 ± 2.4 A
60 kg N ha ⁻¹	7.7 ± 2.2 B	6.3 ± 1.9 B	0.7 ± 0.2 AB	0.6 ± 0.2 A	136.9 ± 80.2 A	102.7 ± 34.9 B	8.2 ± 3.0 B	7.7 ± 3.0 B
90 kg N ha ⁻¹	10.7 ± 2.6 C	8.9 ± 2.4 C	1.0 ± 0.3 B	0.8 ± 0.3 B	144.2 ± 99.3 A	148.6 ± 44.8 C	12.0 ± 3.9 C	11.6 ± 3.9 C
120 kg N ha ⁻¹	13.2 ± 2.2 D	11.0 ± 2.0 D	1.3 ± 0.4 C	0.8 ± 0.3 B	146.5 ± 94.8 A	184.3 ± 37.3 D	14.9 ± 3.3 D	14.5 ± 3.3 D
150 kg N ha ⁻¹	13.7 ± 2.2 D	11.4 ± 1.8 D	1.4 ± 0.5 C	0.9 ± 0.2 B	157.3 ± 105.2 A	188.0 ± 34.0 D	15.0 ± 3.0 D	14.6 ± 3.0 D
Statistics (<i>P</i> values)								
Year	0.0853	<0.05	<0.0001	<0.0001	<0.0001	<0.05	<0.05	<0.05
Site	0.7571	0.7656	0.9303	0.4420	<0.0001	0.7656	0.7656	0.764
Nitrogen	<0.0001	<0.0001	<0.0001	<0.0001	0.7235	<0.0001	<0.0001	<0.0001
Year × Site	<0.0001	<0.0001	0.6000	<0.05	0.7400	<0.0001	<0.0001	<0.0001
Year × Nitrogen	0.2044	0.3092	<0.01	<0.01	0.2551	0.3092	0.3092	0.3088
Site × Nitrogen	0.5933	0.5585	0.7742	0.0897	0.4620	0.5585	0.5585	0.5590
Year × Site × Nitrogen	0.4399	0.4720	0.3456	<0.05	0.3985	0.4720	0.4720	0.4721

washed from the seedbed. During the early part of the growing season, plant height and leaf chlorophyll decreased with delay in the application of nitrogen. Early applied nitrogen stimulated leaf chlorophyll content and early growth although this imbalance was redressed later in the growing season when there was no difference in height between treatments and the highest chlorophyll concentrations were found in the late application treatment. Late nitrogen did stimulate late season LAI and final harvest leaf yield in 1 year of the study (2009), but these differences were not translated into differences in yield possibly because they occurred after flowering when the energy of the plant is focussed on reproduction rather than on growth. However, applying nitrogen at sowing avoids the expense of later or split applications of fertilizer. It would appear that what is important for hemp is that the crop has sufficient supplies of available nitrogen early in the growing season. The principal of early nitrogen application should also apply when organic fertilizers are used to fertilize hemp crops; organic fertilizers such as liquid manure have been used successfully to fertilize hemp (Bosca & Karus, 1997).

Energy and greenhouse balance are critically important when a crop is grown for energy. Nitrogen is typically the most important component in such calculations as the manufacture and application of nitrogenous fertilizers is both energy and GHG intensive (Wood & Cowie, 2004; IPCC, 2006). A significant part of the GHG budget associated with crop production comes from the release of nitrous oxide from soil. Nitrous oxide emissions from soil can exhibit considerable variability depending on nitrogen rate, soil type, form of nitrogen applied and weather (Granli & Bøckman, 1994; de Klein *et al.*, 2001; Rafique *et al.*, 2011). However, the most significant factor is nitrogen rate and both methods of soil N₂O calculation produced similar responses of net GHG emissions to nitrogen application primarily because of the relatively low nitrogen application rates needed to fertilize hemp crops. The application of nitrogenous inorganic fertilizer can be expected to have a positive effect on the energy and GHG balances of a hemp crop grown for biomass up to an application rate of 120 kg N ha⁻¹ above which no further increases can be expected. In comparison to other arable crops, hemp appears to have a low requirement for nitrogen (Hay & Walker, 1989; Coulter & Lalor, 2008). Hemp is a good candidate energy crop as high biomass yields can be produced using relatively low inputs. Energy crops can successfully be used to mitigate GHG emissions (Clifton-Brown *et al.*, 2007). The potential of hemp to mitigate GHG emissions can be maximized by choosing late maturing varieties to produce high yields of biomass and by using a nitrogen application rate of 120 kg N ha⁻¹ applied at sowing.

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