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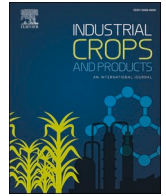
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# Multi-environment assessment of a yellow hemp (*Cannabis sativa* L.) cultivar's eco-physiology and productivity under varying levels of nitrogen fertilisation

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## ABSTRACT

Yellow stalked cultivars of hemp (*Cannabis sativa* L.) were reported to have a superior fibre processability than green cultivars, but the effects of the yellow trait on productivity and ecophysiology of hemp remain unexplored. It is hypothesised that yellow stalked cultivars are characterised by a low Nitrogen Uptake Efficiency. Three field scale experiments involving a yellow cultivar ('Fibror 79') and a green one ('Futura 75') grown under varying levels of nitrogen fertilisation were conducted in Northern Italy and in Wales, to investigate the effects of the yellow trait on hemp productivity and eco-physiology. The results showed that 'Fibror 79' displays a slightly lower biomass productivity than 'Futura 75' under nitrogen-limiting conditions, owing to lower Leaf Area Index and light interception capacity during the early growth phase, and potentially due to a lower radiation use efficiency. However, the productivity was not significantly different between cultivars under non-limiting nitrogen conditions. The Nitrogen Uptake Efficiency did not differ between the two cultivars, which failed to support the hypothesis of this work. Instead, Nitrogen Use Efficiency and Nitrogen Utilisation Efficiency were significantly higher for 'Futura 75' than for 'Fibror 79' under nitrogen-limiting conditions. 'Futura 75' appeared better suited than 'Fibror 79' for dual- and multi-purpose production in Italy, while the reverse was true in Wales. 'Fibror 79' better suits a strategy of single-use fibre production owing to its higher bast fibre content and fibre yield.

## 1. Introduction

Nitrogen is an essential element for plant growth and its intensive use in fertilisers during the second half of the 20th century has led to large increases in crop yields (Zhang et al., 2015). However, the resulting nitrogen losses in the environment have also disturbed its biogeochemical cycle (Fowler et al., 2013) and have resulted in air and water pollution (Erisman et al., 2013). Furthermore, its fixation through the Haber-Bosch process is energy intensive and leads to high CO<sub>2</sub> emissions (Smith et al., 2020). Optimizing nitrogen fertilisation has therefore become a major challenge of modern agriculture. The integration of nitrogen-efficient crops in agricultural systems is a pertinent lever for action for limiting overall nitrogen use, thus minimising the carbon

footprint of agriculture and abate the disturbance of the biogeochemical nitrogen cycle.

Despite being a C3 crop, hemp (*Cannabis sativa* L.) has a relatively low nitrogen demand for biomass or fibre production (Tang et al., 2017a) resulting from its low structural nitrogen and high leaf Photosynthetic Nitrogen Use Efficiency (Tang et al., 2017a, 2017b). Although hemp's acreage has recently risen in the EU (Eurostat, 2021), the development of the hemp sector remains limited by several bottlenecks, such as a lack of dedicated primary transformation centres and limited harvesting mechanisation (Amaducci et al., 2008). In addition, the limited profitability of hemp cropping reduces the willingness of producers to grow it (Meynard et al., 2013). In this regard, cultivating hemp for dual- or multi-purpose production of stems, seeds, and inflorescences

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would maximise the economic potential of the crop. The production of high-quality fibre can also increase hemp profitability, although it seems incompatible with dual- and multi-purpose production as the quality of the fibre decreases during seed formation (Westerhuis et al., 2019). Low-quality fibre is generally used for low-value applications such as building materials or paper pulp production (van der Werf et al., 1994; Crini et al., 2020). In contrast, a high-quality fibre can be used as feedstock for producing high added-value applications such as bio-based materials (Musio et al., 2018) or textiles for clothing applications (Vandepitte et al., 2020). The development of cultivars producing high-quality fibre is therefore an important target of breeding activities (Salentijn et al., 2015; Thouminot, 2015).

In this context, a “yellow” phenotype appears of interest. Discovered by Hoffmann (1946), the “yellow stem” phenotype (originally referred to as “*Hellstengeligen*”) arose from a cross between an Italian and a Finnish landrace. Although evidence is scarce, several authors have reported that yellow-stalked hemp cultivars (YSCs) display a higher stem processability (i.e. fibre extraction efficiency) than conventional green cultivars (CGCs) (Liberalato, 2003; Toonen et al., 2004; Bennett et al., 2006; van den Broeck et al., 2008; Salentijn et al., 2015; Thouminot, 2015; Musio et al., 2018). Thus, the introgression of this trait is becoming a popular target in hemp breeding programs and has led to a significant number of registered “yellow” cultivars such as ‘Carmaleonte’, ‘Ivory’, ‘Kompolti Sgárgaszárdi’ or ‘Chamaeleon’.

Some of these YSCs were also reported to display a higher ratio of long to total fibre (Bennett et al., 2006), a higher bast fibre content and finer hackled fibre bundles than CGCs (Musio et al., 2018). Berenji et al. (2013) and Thouminot (2015) attributed the easier stem processability of “yellow lines” to a reduced efficiency of nitrogen assimilation, but so far this has not been directly confirmed. However, a decreased Nitrogen Uptake Efficiency (NUpE – kg of nitrogen taken up per kg of available soil nitrogen) could explain the yellowness through a relatively low nitrogen content in the biomass and subsequently, through a relatively low chlorophyll content, these two parameters being closely related (Lawlor et al., 2001; Wang et al., 2014). Interestingly, negative effects of increasing levels of nitrogen fertilisation on fibre quality traits (e.g. stem processability for fibre extraction) have been reported (Ehrensing, 1998; Maľceva et al., 2011; Legros et al., 2013; Fike, 2016) and the hypothesis of a low nitrogen content in YSCs is consistent with both their yellowness and the high quality parameters of their fibre.

However, there is a potential drawback of YSCs as their low NUpE could reduce their productivity, as suggested by Thouminot (2015). It has been reported in hot and dry conditions that the YSC ‘Ivory’ produced significantly less biomass than the CGC ‘Santhica 27’, but ‘Ivory’ displayed a faster decline of leaf area index through earlier senescence, and an earlier cessation of stem elongation than ‘Santhica 27’, indicating that a difference of phenology between the two cultivars has probably driven the difference of productivity between the two cultivars (Herppich et al., 2020). In contrast, Cosentino et al. (2013) reported that the YSC ‘Chamaeleon’ had a similar productivity than the CGCs ‘Félina 34’, ‘Futura 75’, and ‘Fédora 17’ in an environment in which all four cultivars had a similar phenology. Similarly, the stem productivity of ‘Markant’ (YSC) and ‘Fédora 17’ (CGC) did not differ significantly in France and Italy, where these two cultivars had a similar phenological development (Tang et al., 2016).

The combination of the hypotheses of a relatively low NUpE displayed by YSCs and of yield similarities between YSCs and CGCs of similar time of flowering would imply that YSCs are also characterized by a higher Nitrogen Utilisation Efficiency (NuTE – kg of produced biomass per kg of nitrogen taken up), in line with the regularly reported increases of NuTE at decreasing levels of nitrogen availability (Ortiz-Monasteiro et al., 1997; Le Gouis et al., 2000; Lawlor et al., 2001; Lemaître et al., 2008).

By comparing two hemp cultivars of similar time of flowering but differing in their phenotype (i.e. a YSC: ‘Fibror 79’ – and a CGC: ‘Futura 75’), the first objective of this work is to characterize the relative

productivity and the putative differences of Nitrogen Use Efficiency (NUE), NUpE and NuTE between ‘Fibror 79’ and ‘Futura 75’, with the hypotheses that ‘Fibror 79’ displays similar productivity and NUE, a lower NUpE and a higher NuTE than ‘Futura 75’. A second objective will be to assess the variability of eco-physiological responses to diverse levels of nitrogen fertilisation between these two cultivars to achieve a better understanding of the implications of the yellow trait on hemp’s productivity and nitrogen management.

## 2. Materials and methods

### 2.1. Plant material, environments, and experimental layout

The experiment was replicated three times: in 2020 and in 2021 at the experimental farm of the Università Cattolica del Sacro Cuore di Piacenza (Northern Italy, 45° 00′ 12″ N, 10° 42′ 13″ E) and in 2021 at the experimental farm on the Gogerddan Campus of Aberystwyth University (Wales, 52° 26′ 03″ N, 4° 01′ 04″ W). These three environments will thereafter be referred to as PC20, PC21, and AB21, respectively. Köppen-Geiger classification’s climate types are Cfa and Cfb for Piacenza and Aberystwyth, respectively. Two commercial cultivars of hemp (*Cannabis sativa* L.) were tested in this study: the YSC ‘Fibror 79’ and the CGC ‘Futura 75’, both cultivars having been bred and provided by Hemp-it (France). ‘Fibror 79’ flowers about four days later than ‘Futura 75’ (Breeder’s description of the cultivars). Sowing was carried out on the 6th and 9th of April in PC20 and PC21, respectively, and on the 27th of April in AB21, with an experimental seed drilling machine (1.5 m wide, seven rows at 21 cm spacing at PC20 and PC21, and 9 rows at 12 cm spacing in the centre of a 1.55 m wide plot in AB21). Sowing depth was about 3 cm, and sowing density was of about 45 kg ha<sup>-1</sup>, targeting 200 viable seeds per square meter based on the one-thousand seed weight and germinability of each seed lot. Four levels of nitrogen were applied: 0, 25, 50, and 100 kg<sub>N</sub> ha<sup>-1</sup>, and were provided by hand with ammonium nitrate 26%, within 24 h before sowing in PC20 and PC21, and the 8th of June (about a month after emergence) in AB21. The nitrogen fertilisation was delayed in AB21 to reduce nitrate leaching during a period of important rainfalls that occurred between sowing and early June. The experimental layout was a complete randomised block design with four levels of nitrogen, two cultivars, and four blocks, for a total of 32 plots in each experimental site. Each plot’s area was 60 m<sup>2</sup> (6 × 10 m) in PC20 and PC21, while it was of 39 m<sup>2</sup> (4.6 × 8.4 m) in AB21. Weather data (average temperature and rainfall) were recorded hourly by a meteorological station situated within 0.5 km from the experiments. Soil characteristics are displayed in Table 1 and all three environments were stone free.

**Table 1**

Soil characteristics measured in the upper 0 – 30 cm depth soil profile. Soil bulk density in PC20 and PC21 was obtained from the study of Maris et al. (2021) which was carried out on the same site, while soil bulk density was retrieved from LUCAS ESDAC topsoil database (Orgiazzi et al., 2018) in AB21. Soil mineral nitrogen was calculated as described in Section 2.2.4. n.a.: not available.

Parameter	PC20	PC21	AB21
Silt (%)	44.8	44.8	41.7
Clay (%)	41.6	41.6	14.8
Sand (%)	13.6	13.6	43.5
Soil total carbon (%)	1.37	1.63	n.a.
Soil total nitrogen (%)	0.17	0.16	n.a.
N-NO <sub>3</sub> (mg kg <sup>-1</sup> )	15.21	9.28	11.60
N-NH <sub>4</sub> (mg kg <sup>-1</sup> )	3.33	2.81	5.10
Bulk density (kg dm <sup>-3</sup> )	1.31	1.31	1.10
Soil mineral nitrogen (kg <sub>N</sub> ha <sup>-1</sup> )	145.7	95.0	125.0

## 2.2. Measurements

### 2.2.1. Emergence date

Emergence date was determined as the day on which 50% of the final number of emerged plants had appeared. The number of emerged plants was measured daily from sowing to the moment at which no plant had newly emerged for three consecutive days, on four one-meter lines per cultivar and per environment. Emergence occurred on the 14th and on the 20th of April in PC20 and PC21, respectively, and on the 11th of May in AB21, and required 112 °Cd, 88 °Cd, and 98 °Cd of accumulated thermal time for completion in PC20, PC21, and AB21, respectively, using a base temperature of 1 °C. Both cultivars emerged on the same day in all three environments.

### 2.2.2. Biomass productivity

Each plot was sampled six times during the growing season (five times in AB21), the sampling times being thereafter referred to as “S1” to “S6”. S1 was set at canopy closure, S5 and S6 were carried out at full-flowering (code 2302 – [Mediavilla et al., 1998](#)) and at seed maturity (code 2306), respectively. S2, S3, and S4 (only S2 and S3 in AB21) were regularly spaced between S1 and S5. Dates and thermal time accumulation of each sampling time is reported in [Supplementary material S1](#). At each sampling time and in each plot, a quadrat of one square metre was placed, keeping a distance of at least one metre from the plot border or from areas that had previously been sampled to avoid border effects. In all three environments, all plants within the quadrat were cut at ground level, counted, and immediately weighed for fresh weight determination of aboveground biomass. Ten representative plants surrounding the quadrat (or within the quadrat at AB21) were concomitantly sampled, the leaves were separated from the stems and each fraction was immediately weighed for fresh weight determination. The samples were subsequently oven-dried at 105 °C until constant weight for determining the biomass partitioning and the water content of each biomass fraction. The dry biomass weight of the quadrat was estimated from the fresh weight of the plants harvested in the quadrat and from the water content of the samples. Quadrat stem biomass was calculated as the product of the quadrat dry weight (obtained after fitting procedure, see [Section 2.3.1.](#)) and of the stem fraction of the dry biomass, which was estimated from biomass partitioning values of the samples. Total biomass and its components were then converted to t ha<sup>-1</sup>. At S6 after drying, inflorescences (leaves, flowers, and seeds) of all plants in each quadrat were manually separated from the stems, and seeds were separated from the threshing residues (leaves and flowers) using experimental threshers (PC20 and PC21: Small Thresher Plot, model 2375, Cicoria Company, San Gervasio, Italy; AB21: locally made thresher) to obtain the yield of each fraction.

To determine the fibre yield, five representative stems per plot were sampled at full-flowering and seed maturity in all three environments. The stems were dried at room temperature for at least three months. Each stem was cut at 10 and 110 cm from its base to obtain a 100 cm long stem sample which was weighed and processed six times in a laboratory scale decorticator (Wortmann Maschinenbau GmbH, Barßel-Harkebrügge, Germany). After that, bast fibre was manually cleaned to remove attached pieces of shives and weighed. Bast fibre content was calculated as the ratio of the weight of the cleaned bast fibre to that of the stem sample before processing. Fibre yield was obtained by multiplying the stem yield by the average bast fibre content, assuming a uniform bast fibre content along the whole length of the stem.

### 2.2.3. Leaf area index, light interception and light absorbance

In PC20 and PC21, light interception measurements were carried out in the quadrat, prior to sampling, under clear sky conditions. When the sun reached the zenith (between 12:00 and 14:00), the Photosynthetically Active Radiation (PAR) transmitted by the canopy (PAR<sub>t</sub>) was measured five or six times, at ground level, with a ceptometer (AccuPAR LP-80, Decagon Devices, Inc., Pullman, Washington, USA). An

externally mounted sensor was used to measure incident PAR (PAR<sub>i</sub>). The fraction of PAR transmitted by the canopy (τ<sub>0</sub>) was calculated as the ratio of PAR<sub>t</sub> / PAR<sub>i</sub>. When conditions were cloudy on the date of destructive sampling (which occurred at S3 in both years), measurements of light interception were delayed to the 9th of July in PC20 and to the 1st of July in PC21.

Leaf Area Index (LAI) and Specific Leaf Area (SLA) were determined in PC20 and PC21 during vegetative growth. At samplings S1 to S4, three to five representative plants adjoining the quadrat were sampled. To obtain enough material for subsequent analyses, the number of plants sampled was higher for earlier samples. All leaves were carefully separated from the stems, placed in zip sealed plastic bags, kept flat, and quickly transported to a – 18 °C freezer where they remained until leaf area determination. All leaves were scanned (EPSON Expression 10,000 XL) and leaf area was determined from the scans using R (v 4.1.1., packages ‘EBImage’, ‘nnet’, ‘NeuralNetTools’). The leaves were subsequently oven-dried at 65 °C to constant weight and weighed with a 0.01 mg precision scale. SLA (cm<sup>2</sup> mg<sup>-1</sup>) was calculated as the ratio of leaves area to their dry weight and LAI (m<sup>2</sup> m<sup>-2</sup>) was subsequently estimated according to [Eq. 1](#).

$$LAI = DW_Q \times f_l \times SLA \times 0.1 \quad (1)$$

Where DW<sub>Q</sub> is the quadrat dry weight (g m<sup>-2</sup>), f<sub>l</sub> is the leaf fraction of the dry quadrat biomass (estimated from biomass partitioning values of the 10 plants samples) and SLA is the Specific Leaf Area (cm<sup>2</sup> mg<sup>-1</sup>).

In AB21, leaf absorbance between 400 and 700 nm was measured on leaves sampled from each plot at S2 using a Spectroclip (JAZ-TR, Ocean Optics, Oxford, UK). Measurements were made on five leaflets wide enough to accommodate the measuring head whilst avoiding the midrib on two leaves from each of the three upper layers of the canopy (100–90%, 90–75% and 75–60% of canopy height, 0% representing ground level and 100% representing the average canopy height) and averaged for each layer. As leaves in the lower layers of the canopy (60–0% of canopy height) were visibly senescing, five measurements per leaf were made on one leaf from each of the darker, mid colour and light-coloured sub layers and averaged for each sub-layer. The classification of leaves’ colour was realised based on operator’s experience. Reflectance (R%) was standardised against a calibrated Spectralon reference (SRT-99-020, Labsphere, Sutton, New Hampshire, USA) after correction for the dark reading. Transmission (T%) was calculated by reference to the absence of a sample and a dark reading. Absorbance (A%) was calculated as 100-T-R. Further details on the method are reported in [Supplementary material S2](#).

### 2.2.4. Nitrogen use, uptake, and utilisation efficiencies

In all three environments at S5, three representative plants per plot were sampled for nitrogen content determination in stems and inflorescences (stems, leaves, and flowers in AB21). In Italy, each fraction was oven-dried at 65 °C to constant weight and ground to particle size < 2 mm using a grindomix (Grindomix GM 200, Retsch, Germany). Nitrogen content of each fraction was measured on samples of about 0.5 g of dry matter, using a CN analyser (Vario Max CN Analyzer, Elementar Americas Inc., Hanau, Germany). At AB21, samples were freeze dried and fine milled (< 100 μm for leaves and flowers < 400 μm for stems) using a robot mill (IBER, Labman Automation Ltd. Seamer Hill, Stokesley, North Yorkshire, TS9 5NQ, UK) then 2–4 mg were precisely weighed and analysed for % nitrogen by weight using a continuous-flow isotope-ratio mass spectrometer (ANCA-SL 20–20, Europa Scientific, Crewe, UK).

The Nitrogen Use Efficiency (NUE) was calculated as the dry biomass produced at full-flowering per amount of soil available nitrogen and expressed in kg<sub>DM</sub> kg<sub>N</sub><sup>-1</sup>. Soil available nitrogen was calculated as nitrogen supplied by fertilisation plus soil mineral nitrogen ([Gaju et al., 2011](#)) in the 0–60 cm top-soil layer. In Italy, soil mineral nitrogen was estimated from samples taken within 24 h prior to fertilisation in the 0 –

30 cm topsoil layer, in each plot. Soil samples were kept frozen at  $-18^{\circ}\text{C}$  prior to analysis, pooled per bloc, and analysed in triplicates for determining the contents of  $\text{N-NO}_3$  and  $\text{N-NH}_4$ , following the methods described in Kaneko et al. (2010) and Rhine et al. (1998), respectively. In AB21, five cores were taken in the 0–10 cm layer, from 10 areas in the experimental field. The five cores were pooled (for each of the ten areas) and the ten frozen soil samples were sent to, and analysed by NRM laboratories (NRM, Coopers Bridge, Braziers Lane, Bracknell, Berkshire RG42 6NS, England) for  $\text{N-NO}_3$  and  $\text{N-NH}_4$  contents measurements. To determine the total amount of soil mineral nitrogen in the 0–60 cm top-soil layer, the concentrations of  $\text{N-NO}_3$  and  $\text{N-NH}_4$  measured in the soil samples were summed and multiplied by soil bulk density (Table 1). The Nitrogen Uptake Efficiency (NUpE) was calculated as the amount of nitrogen taken up by the crop at S5 per kg of soil available nitrogen and expressed in  $\text{kg}_\text{N} \text{kg}_\text{N}^{-1}$ . The amount of nitrogen taken up by the crop was calculated by multiplying the biomass of each fraction (stems and leaves) by their respective nitrogen contents and by adding the two terms. The Nitrogen Utilisation Efficiency (NuTE) was calculated as the dry biomass produced per unit of nitrogen taken up at S5 and expressed in  $\text{kg}_\text{DM} \text{kg}_\text{N}^{-1}$ . NUE, NUpE, and NuTE were measured at full-flowering (S5) rather than at seed maturity (S6) to avoid high variability in results due to biomass losses during seed maturation (due to e.g. self-thinning, seed shattering, leaf losses etc.), and because YSCs are here considered of interest for their fibre quality, which is higher at full-flowering than at seed maturity (Westerhuis et al., 2019).

### 2.3. Data processing

#### 2.3.1. Biomass dynamics

In order to limit the variance induced by inherent *in field* variability, a Schnute equation (Schnute, 1981; Barillot et al., 2012) was fitted to the aboveground biomass measured in field (Eq. 2) for each plot and in each environment, for a total of ninety-six fittings, using R (v. 4.1.1., package ‘nls.multstart’).

$$W(t) = \left( \frac{W_{\max}^B \times (1 - \exp(-A \times t))}{1 - \exp(-t_{\max} \times A)} \right)^{\frac{1}{B}} \quad (2)$$

With  $W(t)$  being the biomass produced ( $\text{t ha}^{-1}$ ) as a function of accumulated thermal time  $t$  ( $^{\circ}\text{Cd}$ ), using a base temperature of  $1^{\circ}\text{C}$  (Tang et al., 2016).  $W_{\max}$  is the biomass produced at  $t = t_{\max}$ ,  $t_{\max}$  being the value of thermal time accumulation between emergence and S6 (Supplementary material S1), and it was treated as an independent variable that was fixed for each cultivar and each environment of cultivation.  $A$  and  $B$  are two curvature parameters without biological meaning. Because biomass fittings tended to underestimate the observed biomass productivity in early growth stages, the values of measured biomass at S1 used as an input for the fitting procedure were triplicated to force the fittings to properly describe the whole growing season. The biomass productivity at S5 and S6 was then determined from each fitting using the corresponding set of parameters  $\{W_{\max}; A; B\}$  and the value of thermal time accumulated at each sampling, and these values were used instead of those measured *in field*. Each fitting’s quality was assessed through the study of the NRMSE (defined as the RMSE normalised by the maximal value of measured biomass of the dataset considered), through the study of the multiple R-squared between measured and fitted values, by assessing normality of distribution of residuals using the Shapiro-Wilk test ( $\alpha = 0.05$ ), and by graphical analysis. These quality assessments revealed that two datasets out of ninety-six had an outlier which was removed before re-fitting. Additional fittings using the Schnute function were realised on the data of biomass averaged across all levels of nitrogen for each genotype, and on the data of biomass averaged between both cultivars for each level of nitrogen fertilisation, for the purpose of data presentation (see Section 3.2.).

#### 2.3.2. Light extinction coefficient

Light extinction curves were fitted using exponential relationship between  $\tau_0$  and LAI (Eq. 3; Meijer et al., 1995; Cosentino et al., 2013; Tang et al., 2018) for each plot in PC20 and PC21.

$$1 - \tau_0 = 1 - \exp(-\kappa_l \times \text{LAI}) \quad (3)$$

Where LAI is the Leaf Area Index (as described in Section 2.2.3.),  $\kappa_l$  is the light extinction coefficient whose value was computed using R (v. 4.1.1., package ‘nls.multstart’), and  $1 - \tau_0$  is the fraction of PAR intercepted by the canopy (see Section 2.2.3.). The quality of the fittings was assessed through the study of the RMSE, of the multiple R squared between measured and fitted values of light interception, of the normality of distribution of the residuals, assessed with the Shapiro-Wilk test ( $\alpha = 0.05$ ), and by visual analysis of the graphs.

### 2.4. Statistical analysis

The effects of genotype, nitrogen, and of their interaction on the parameters were assessed by two-way anova. Post-hoc analyses used Tukey’s HSD test ( $\alpha = 0.05$ ). The assumptions of normality of distribution of residuals and of homoscedasticity were assessed with the tests of Shapiro-Wilk and Levene ( $\alpha = 0.05$ ), respectively. For each parameter assessed, a maximum of one outlier per environment was removed to comply with these assumptions. The data are presented as means  $\pm$  standard error. All statistical analyses were carried out using R (v. 4.1.1., packages ‘agricolae’, ‘car’).

## 3. Results

### 3.1. Environmental and crop characteristics

Daily mean temperatures ranged from  $11.4^{\circ}\text{C}$  (8th of April) to  $29.3^{\circ}\text{C}$  (1st of August) in PC20, from  $6.7^{\circ}\text{C}$  (16th of April) to  $28.3^{\circ}\text{C}$  (13th of August) in PC21 (Fig. 1), and from  $5.0^{\circ}\text{C}$  (30th of April) to  $22.4^{\circ}\text{C}$  (22nd of July) in AB21. Rainfall was  $295.6 \text{ mm}$  in PC20,  $258.4 \text{ mm}$  in PC21, and  $456.4 \text{ mm}$  in AB21. Overall, rainfall did not compensate for evapotranspiration in Italy and irrigation was carried out four times in PC20 and three times in PC21, providing respectively  $100 \text{ mm}$  and  $105 \text{ mm}$  of water (Supplementary material S1). No irrigation was performed in AB21. Plant density measured at S1 was  $196 \pm 5 \text{ plants m}^{-2}$  in PC20 and  $183 \pm 3 \text{ plants m}^{-2}$  in PC21 and  $183 \pm 9 \text{ plants m}^{-2}$  in AB21, with no significant differences between genotypes (statistics not shown). In all environments, plant density progressively decreased during the growing season to reach  $144 \pm 3$ ,  $114 \pm 3$ , and  $142 \pm 6 \text{ plants m}^{-2}$  at seed maturity in PC20, PC21, and AB21, respectively, with no significant differences between genotypes (statistics not shown).

Crops did not suffer from diseases or weeds. Pests (e.g. *Chinavia hilaris*, *Ostrinia nubilalis*) were present in PC20 and PC21, but infestation levels were low and not expected to have any significant effect on aboveground biomass productivity. Crop phenology was similar between the two cultivars in all three environments with much greater thermal times accumulated to flowering maturity in PC20 and PC21 than in AB21 (Supplementary material S1). ‘Fibror 79’ was of a slightly lighter green than ‘Futura 75’ after emergence and this difference of colour between the two cultivars tended to increase during the growing season, to become a dark yellow and a dark green at seed maturity for ‘Fibror 79’ and ‘Futura 75’, respectively (Supplementary material S3, Impollonia et al., 2022).

### 3.2. Biomass productivity at full-flowering and at seed maturity

All fittings of biomass dynamics obtained with Eq. 2 described the measured data of aboveground biomass productivity relatively well. Across all ninety-six fittings, the values of multiple-R squared between

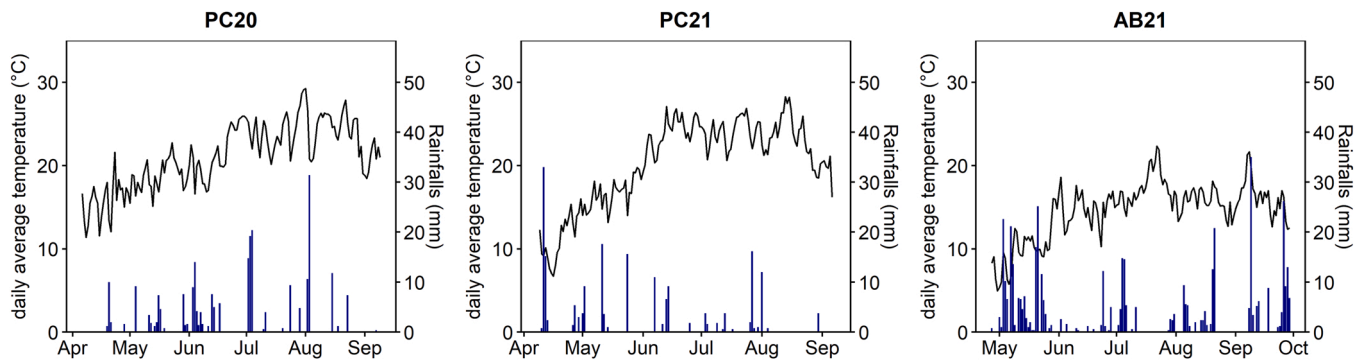


Fig. 1. Daily average temperature (continuous line) and rainfall (blue bars) in the three environments of study between sowing and S6.

observed and fitted values of biomass ranged from 0.84 to 0.99, and NRMSE ranged from 0.73% to 12.7% (Supplementary material S4). Nine fittings did not have a normal distribution of residuals, which was mostly caused by outliers at the end of the growing season, as the variability between replicates often increased with age (Supplementary material S5).

Across all conditions of genotype, nitrogen fertilisation, harvest time, and environment, aboveground biomass productivity ranged from  $7.4 \pm 0.4 \text{ t ha}^{-1}$  for unfertilised 'Fibror 79' sampled at full-flowering in PC20, to  $14.9 \text{ t ha}^{-1}$  for 'Fibror 79' fertilised with  $100 \text{ kg}_N \text{ ha}^{-1}$  and sampled at seed maturity in AB21 (Supplementary material S6). Generally, crop productivity was similar between the two cultivars (Fig. 2), as the genotype effect was not significant in AB21 and PC21 (Table 2), and as the difference did not exceed  $0.6 \text{ t ha}^{-1}$  in these two environments at either harvest time (Table 3). However, at PC20, aboveground biomass productivity was significantly affected by genotype both at full-flowering and seed maturity (Table 2), as 'Futura 75'

produced about  $1.5 \text{ t ha}^{-1}$  more than 'Fibror 79' in either harvest time (Fig. 2, Table 3). Similarly, nitrogen fertilisation had a significant effect on crop productivity in PC20 but not in the other two environments (Fig. 2, Tables 2 and 3), although increasing levels of nitrogen fertilisation tended to lead to biomass increments in AB21, in particular at seed maturity (Fig. 2, Table 3). The interaction between genotype and nitrogen fertilisation was not significant in any combination of environment and sampling time (Table 2).

Stem productivity was not affected by the genotype in either combination of environment and harvest time but was significantly affected by nitrogen fertilisation at both harvest times in PC20 (Table 2). Over all combinations of genotype, nitrogen fertilisation, environment, and harvest time, it ranged from  $5.7 \pm 0.4 \text{ t ha}^{-1}$  at full-flowering in PC20 to  $10.6 \pm 1.3 \text{ t ha}^{-1}$  at seed maturity in AB21, (Supplementary material S6).

Fibre yield ranged from  $1.82 \pm 0.16 \text{ t ha}^{-1}$  for 'Futura 75' fertilised with  $25 \text{ kg}_N \text{ ha}^{-1}$  in PC20 at seed maturity, to  $3.81 \pm 0.71 \text{ t ha}^{-1}$  for

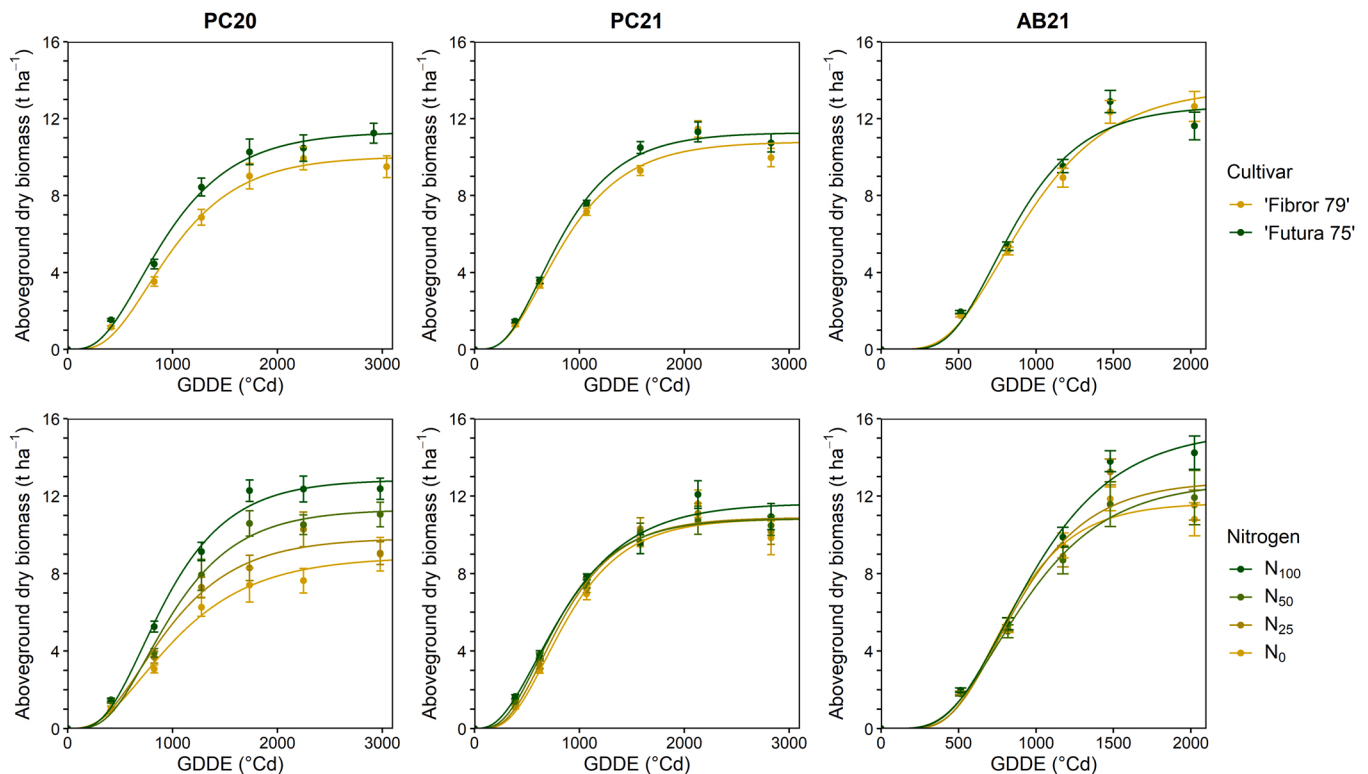


Fig. 2. Dynamics of aboveground dry biomass as a function of thermal time accumulation (base temperature =  $1 \text{ }^\circ\text{C}$ ) from emergence to seed maturity, for each genotype (top) and each level of nitrogen fertilisation (bottom), in the three environments. Points and error bars are the average  $\pm$  standard error of the data measured in field ( $n = 16$  for each genotype and  $n = 8$  for each level of nitrogen fertilisation). The continuous lines are the fittings realised on these averaged values using the Schnute function (Eq. 2). GDDE: growing degree day from emergence.

**Table 2**

p-values of the effects of genotype (G), nitrogen (N) and of their interaction (G × N) on total aboveground biomass productivity, stem productivity and fibre productivity, for each harvest time (full-flowering and seed maturity) and each environment, assessed by two-ways anova. Bold values highlight the significant effects ( $\alpha = 0.05$ ).

Biomass fraction	Effect	PC20		PC21		AB21	
		Full-flowering	Seed maturity	Full-flowering	Seed maturity	Full-flowering	Seed maturity
Total aboveground biomass	G	<b>2.1 e<sup>-3</sup></b>	<b>0.013</b>	0.26	0.35	0.93	0.52
	N	<b>1.5 e<sup>-4</sup></b>	<b>2.5 e<sup>-4</sup></b>	0.84	0.81	0.10	0.12
	G × N	0.69	0.90	0.82	0.76	0.81	0.42
Stems	G	0.15	0.74	0.52	0.30	0.52	0.35
	N	<b>1.5 e<sup>-5</sup></b>	<b>9.7 e<sup>-5</sup></b>	0.70	0.64	0.39	0.28
	G × N	0.97	0.82	0.87	0.85	0.85	0.76
Fibre	G	<b>0.014</b>	<b>7.1 e<sup>-3</sup></b>	<b>2.5 e<sup>-3</sup></b>	<b>1.2 e<sup>-3</sup></b>	<b>0.037</b>	0.093
	N	<b>6.1 e<sup>-6</sup></b>	<b>3.8 e<sup>-3</sup></b>	0.44	0.43	0.51	0.37
	G × N	0.75	0.57	0.68	0.86	0.75	0.55

**Table 3**

Productivity of total aboveground biomass, stem, and fibre (t ha<sup>-1</sup>), at full-flowering and at seed maturity, in all three environments, for each cultivar. Data presented are the mean ± standard error, averaged across all levels of nitrogen fertilisation (n = 16 for each genotype), or between both genotype (n = 8 for each level of nitrogen fertilisation).

Biomass fraction	Factor	Genotype / Nitrogen	PC20		PC21		AB21	
			Full-flowering	Seed maturity	Full-flowering	Seed maturity	Full-flowering	Seed maturity
Total aboveground biomass	Genotype	'Fibror 79'	9.4 ± 0.5	9.9 ± 0.6	10.4 ± 0.3	10.8 ± 0.4	11.4 ± 0.4	13.2 ± 0.7
		'Futura 75'	10.9 ± 0.5	11.4 ± 0.5	11.0 ± 0.3	11.4 ± 0.4	11.4 ± 0.4	12.6 ± 0.6
	Nitrogen fertilisation	N <sub>0</sub>	8.4 ± 0.8	9.0 ± 0.8	10.5 ± 0.6	10.9 ± 0.7	10.8 ± 0.4	11.8 ± 0.6
		N <sub>25</sub>	9.1 ± 0.6	9.5 ± 0.6	10.6 ± 0.4	11.0 ± 0.4	11.4 ± 0.5	12.6 ± 0.6
		N <sub>50</sub>	10.8 ± 0.5	11.4 ± 0.5	10.6 ± 0.4	10.9 ± 0.5	10.7 ± 0.8	12.5 ± 1.4
Stem	Genotype	'Fibror 79'	7.3 ± 0.4	7.5 ± 0.5	7.7 ± 0.2	8.1 ± 0.3	8.7 ± 0.4	9.4 ± 0.5
		'Futura 75'	8.0 ± 0.4	7.7 ± 0.5	7.9 ± 0.2	8.6 ± 0.3	9.1 ± 0.4	9.7 ± 0.5
	Nitrogen fertilisation	N <sub>0</sub>	6.2 ± 0.6	6.1 ± 0.6	7.6 ± 0.4	8.0 ± 0.5	8.6 ± 0.4	9.2 ± 0.6
		N <sub>25</sub>	6.8 ± 0.5	6.5 ± 0.5	7.7 ± 0.3	8.3 ± 0.3	8.9 ± 0.5	9.5 ± 0.6
		N <sub>50</sub>	8.1 ± 0.3	8.1 ± 0.5	7.6 ± 0.3	8.4 ± 0.4	8.4 ± 0.8	9.3 ± 1.0
Fibre	Genotype	'Fibror 79'	2.7 ± 0.2	2.6 ± 0.2	2.7 ± 0.1	2.9 ± 0.1	3.1 ± 0.2	3.5 ± 0.2
		'Futura 75'	2.3 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.7 ± 0.1	2.9 ± 0.2
	Nitrogen fertilisation	N <sub>0</sub>	2.1 ± 0.2	1.9 ± 0.2	2.4 ± 0.2	2.5 ± 0.2	2.8 ± 0.1	3.2 ± 0.2
		N <sub>25</sub>	2.2 ± 0.2	2.1 ± 0.2	2.5 ± 0.1	2.7 ± 0.1	2.9 ± 0.2	3.1 ± 0.1
		N <sub>50</sub>	2.6 ± 0.1	2.5 ± 0.2	2.4 ± 0.1	2.6 ± 0.2	2.7 ± 0.3	3.1 ± 0.4
		N <sub>100</sub>	3.2 ± 0.1	2.9 ± 0.2	2.6 ± 0.2	2.8 ± 0.1	3.1 ± 0.2	3.4 ± 0.2

'Fibror 79' fertilised with 50 kg<sub>N</sub> ha<sup>-1</sup> in AB21 at seed maturity. Nitrogen fertilisation had a significant effect on fibre yield in PC20 but not in the other two environments (Table 2). 'Fibror 79' always produced significantly more fibre than 'Futura 75' (Table 3) except at AB21 at seed maturity when this difference was not significant (Table 2). Overall, the higher fibre yield of 'Fibror 79' over 'Futura 75' can mostly be explained by its higher bast fibre content which averaged 36.0 ± 0.2% across all conditions of environment, sampling time and nitrogen fertilisation, against 28.9 ± 0.2% for 'Futura 75' (Supplementary material S7, n = 480).

The biomass of threshing residues was significantly affected by the genotype in PC20 and in AB21, but not in PC21 (Table 4). In PC20, 'Futura 75' produced more threshing residues than 'Fibror 79', while

**Table 4**

p-values of the effects of genotype (G), nitrogen (N) and of their interaction (G × N) on the biomass of threshing residues and seeds, at seed maturity and for each environment, assessed by two-ways anova. Bold values highlight the significant effects ( $\alpha = 0.05$ ).

Biomass fraction	Factor	PC20	PC21	AB21
Threshing residues	G	<b>7.0 e<sup>-8</sup></b>	0.65	<b>7.5 e<sup>-5</sup></b>
	N	0.25	0.53	<b>1.9 e<sup>-3</sup></b>
	G × N	0.71	0.58	0.58
Seeds	G	<b>2.9 e<sup>-7</sup></b>	<b>0.012</b>	0.47
	N	0.13	0.98	<b>6.4 e<sup>-5</sup></b>
	G × N	<b>0.018</b>	0.88	0.40

the reverse occurred in AB21 (Table 5).

Seed productivity was significantly affected by the genotype in PC20 and PC21, where 'Futura 75' produced more seeds than 'Fibror 79', but not in AB21 (Tables 4 and 5). Seed productivity was much greater in AB21 (1.65 ± 0.08 t ha<sup>-1</sup>) than it was in Italy (0.14 ± 0.01 t ha<sup>-1</sup> and 0.32 ± 0.03 t ha<sup>-1</sup> in PC20 and PC21, respectively). Contrary to the total aboveground biomass, seed productivity increased significantly with increasing nitrogen levels in AB21, but not in the other two environments (Table 4).

### 3.3. Leaf Area Index, light interception and light absorbance

In PC20 and PC21, across all conditions of genotype and nitrogen fertilisation, LAI increased continuously from S1 to S4 (Fig. 3). The fraction of light transmitted by the canopy decreased during the early growth phase but reached a plateau from S2 and remained relatively stable during the rest of the vegetative growth (Fig. 3).

In PC20, 'Futura 75' had a significantly higher LAI than 'Fibror 79' during most of the vegetative growth (Table 6, Fig. 3, Supplementary material S8). This resulted in a significantly lower fraction of transmitted PAR through the canopy ( $\tau_0$ ) by 'Futura 75' over 'Fibror 79' at S1 but not at later sampling times (Table 6, Fig. 3, Supplementary material S8). The effect of nitrogen fertilisation on LAI and  $\tau_0$  was significant during the whole vegetative growth (Table 6, Fig. 3, Supplementary materials S8 and S9).

**Table 5**

Productivity of threshing residues and seeds ( $t\ ha^{-1}$ ) at seed maturity, in all three environments, for each cultivar and each level of nitrogen fertilisation. Data presented are the mean  $\pm$  standard error, averaged across all levels of nitrogen fertilisation ( $n = 16$  for each genotype), or between both genotype ( $n = 8$  for each level of nitrogen fertilisation).

Biomass fraction	Factor	Cultivar / Nitrogen	PC20	PC21	AB21
Threshing residues	Genotype	'Fibror 79'	2.3 $\pm 0.1$	2.4 $\pm 0.1$	2.1 $\pm 0.1$
		'Futura 75'	3.6 $\pm 0.1$	2.4 $\pm 0.1$	1.3 $\pm 0.1$
	Nitrogen fertilisation	N <sub>0</sub>	2.7 $\pm 0.3$	2.5 $\pm 0.2$	1.4 $\pm 0.1$
		N <sub>25</sub>	2.9 $\pm 0.3$	2.4 $\pm 0.2$	1.5 $\pm 0.2$
		N <sub>50</sub>	3.1 $\pm 0.3$	2.2 $\pm 0.2$	1.5 $\pm 0.3$
		N <sub>100</sub>	3.0 $\pm 0.2$	2.5 $\pm 0.2$	2.3 $\pm 0.2$
Seeds	Genotype	'Fibror 79'	0.09 $\pm 0.01$	0.24 $\pm 0.04$	1.69 $\pm 0.12$
		'Futura 75'	0.19 $\pm 0.01$	0.41 $\pm 0.04$	1.61 $\pm 0.11$
	Nitrogen fertilisation	N <sub>0</sub>	0.16 $\pm 0.03$	0.35 $\pm 0.08$	1.21 $\pm 0.06$
		N <sub>25</sub>	0.14 $\pm 0.02$	0.33 $\pm 0.06$	1.55 $\pm 0.11$
		N <sub>50</sub>	0.14 $\pm 0.03$	0.32 $\pm 0.05$	1.69 $\pm 0.13$
		N <sub>100</sub>	0.11 $\pm 0.01$	0.30 $\pm 0.08$	2.15 $\pm 0.14$

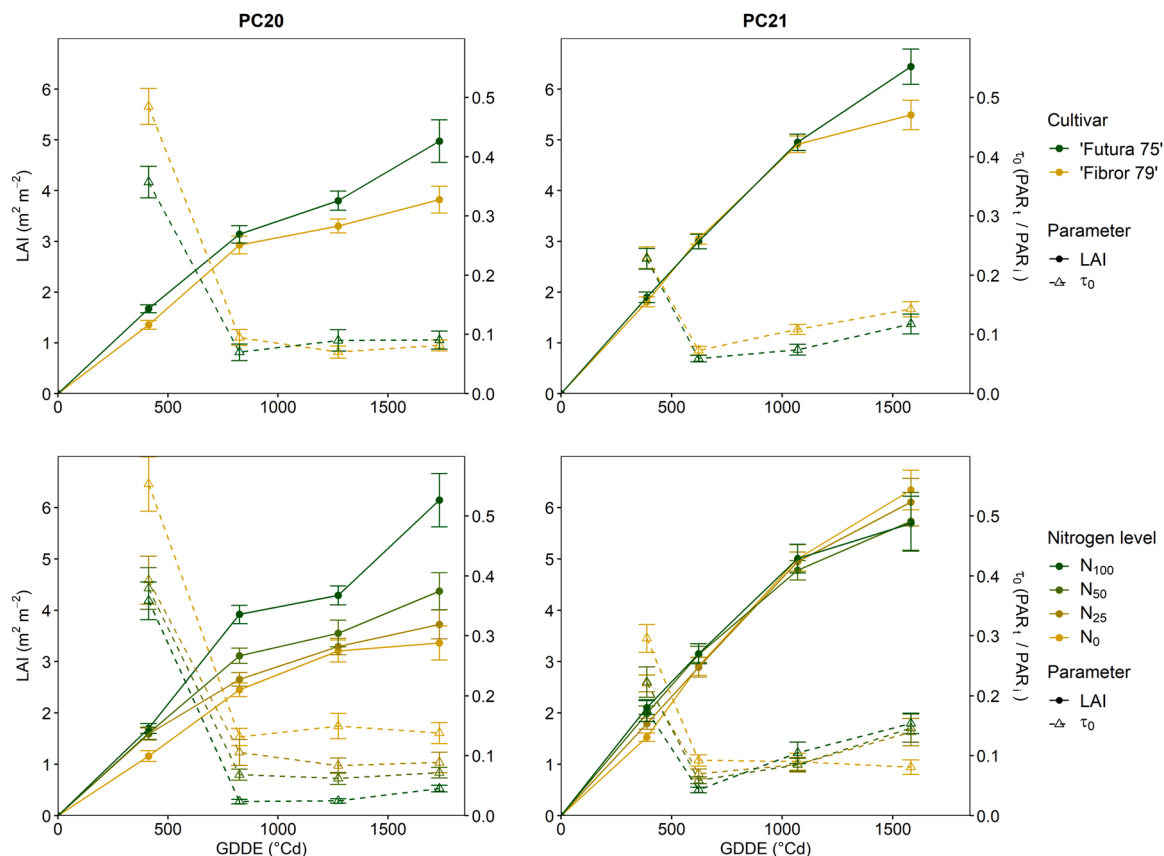
In PC21, both LAI and  $\tau_0$  were not significantly different between the two cultivars except  $\tau_0$  at S3 when 'Fibror 79' transmitted more PAR than 'Futura 75' (Fig. 3, Table 6, Supplementary material S9). Nitrogen fertilisation did have significant effects on LAI at S1 but not subsequently (Table 6, Fig. 3), while it significantly affected  $\tau_0$  at S1, S2, and S4 (Table 6), but in an inconsistent manner and in a lesser extent than in PC20.

Overall, the fraction of PAR intercepted increased with increasing LAI up to about  $2.5\ m^2\ m^{-2}$ , above which it was somewhat variable between 0.8 and 1 (Fig. 4). The light extinction curves were almost identical between both cultivars with averaged  $\kappa_l$  values of  $0.74 \pm 0.02$

**Table 6**

p-values of the effects of genotype (G), nitrogen fertilisation (N) and of their interaction (G  $\times$  N) on the Leaf Area Index and on the fraction of PAR transmitted by the canopy ( $\tau_0$ ) in each environment, for the sampling times S1 to S4 ( $n = 4$ ). Bold values highlight the significant effects ( $\alpha = 0.05$ ).

Sampling time	Factor	PC20		PC21	
		LAI	$\tau_0$	LAI	$\tau_0$
S1	G	<b><math>3.3\ e^{-3}</math></b>	<b><math>4.3\ e^{-4}</math></b>	0.50	0.93
	N	<b><math>3.2\ e^{-3}</math></b>	<b><math>6.5\ e^{-4}</math></b>	<b>0.023</b>	<b><math>5.7\ e^{-3}</math></b>
	G $\times$ N	0.75	0.74	0.84	0.63
S2	G	0.18	0.19	0.77	0.064
	N	<b><math>2.1\ e^{-6}</math></b>	<b><math>3.8\ e^{-5}</math></b>	0.58	<b><math>4.7\ e^{-4}</math></b>
	G $\times$ N	0.92	1.00	0.14	0.70
S3	G	<b>0.022</b>	0.11	0.87	<b>0.013</b>
	N	<b><math>7.1\ e^{-3}</math></b>	<b><math>1.1\ e^{-6}</math></b>	0.90	0.66
	G $\times$ N	0.76	<b>0.038</b>	0.52	0.53
S4	G	<b><math>7.6\ e^{-4}</math></b>	0.80	0.052	0.20
	N	<b><math>3.8\ e^{-6}</math></b>	<b><math>1.5\ e^{-4}</math></b>	0.72	<b>0.042</b>
	G $\times$ N	0.096	0.42	0.38	0.23



**Fig. 3.** Effects of genotype (top) and nitrogen fertilisation (bottom) on the dynamics of LAI (solid lines) and  $\tau_0$  (dashed lines) during the vegetative growth, in PC20 (left) and PC21 (right). Points and error bars are means and their standard errors ( $n = 16$  for genotype effect, and  $n = 8$  for nitrogen fertilisation effect). GDDE: growing degree day after emergence.



for 'Fibror 79' and  $0.76 \pm 0.02$  for 'Futura 75'. No clear effect of nitrogen fertilisation could be observed, as averaged  $\kappa_l$  values were  $0.73 \pm 0.03$ ,  $0.80 \pm 0.05$ ,  $0.74 \pm 0.02$  and  $0.75 \pm 0.02$  for  $N_0$ ,  $N_{25}$ ,  $N_{50}$ , and  $N_{100}$ , respectively.

In AB21, PAR absorbance by leaves was the highest in the top layers of the canopy and decreased with the depth in canopy, following a similar pattern for both cultivars. However, the decrease of absorbance with the increasing depth in the canopy appeared more important for 'Fibror 79' than for 'Futura 75' (Table 7). Indeed, 'Futura 75' had a slightly but significantly higher PAR absorbance than 'Fibror 79', across the top three layers of the canopy but this difference became more important in the lowest layers (Table 7). Overall, nitrogen did not affect the leaf absorbance except in the top and in the bottom layers (Table 7), but these differences across nitrogen treatments showed inconsistent patterns and are not presented.

### 3.4. Nitrogen content and nitrogen use, uptake and utilisation efficiencies

Overall, total nitrogen uptake at full-flowering reached similar values in AB21 ( $92.9 \pm 5.6 \text{ kg}_N \text{ ha}^{-1}$ ) and in PC20 ( $94.4 \pm 5.6 \text{ kg}_N \text{ ha}^{-1}$ ), but this value increased to  $176.8 \pm 5.7 \text{ kg}_N \text{ ha}^{-1}$  in PC21. Total nitrogen found in inflorescences was significantly affected by nitrogen in PC20 (p-value =  $2.5 \times 10^{-4}$ ) and in AB21 (p-value =  $4.0 \times 10^{-7}$ ), where increasing levels of nitrogen fertilisation led to increases of total leaf nitrogen, and by genotype (p-value =  $9.1 \times 10^{-3}$ ) in AB21 where 'Fibror 79' had a higher amount of nitrogen ( $73.8 \pm 4.3 \text{ kg}_N \text{ ha}^{-1}$ ) than 'Futura 75' ( $60.3 \pm 5.9 \text{ kg}_N \text{ ha}^{-1}$ ). In PC21, none of the factors had a significant effect on total leaf nitrogen.

The effects of genotype and nitrogen fertilisation on NUE, NUtE and NUpE varied across the three environments (Fig. 5). In AB21, both NUE and NUtE significantly decreased at increasing levels of nitrogen fertilisation (Table 8) but NUpE was similar at all levels of nitrogen. 'Fibror79' had a significantly higher NUpE ( $0.59 \pm 0.03 \text{ kg}_N \text{ kg}_N^{-1}$ ) than 'Futura 75' ( $0.50 \pm 0.02 \text{ kg}_N \text{ kg}_N^{-1}$ ), while the reverse was true for NUtE (Table 8, Fig. 5, Supplementary material S10), a combination which resulted in a similar NUE between the two cultivars.

In PC20, 'Futura 75' also had a significantly higher NUtE ( $122.5 \pm 6.8 \text{ kg}_{DM} \text{ kg}_N^{-1}$ ) than 'Fibror79' ( $103.6 \pm 4.1 \text{ kg}_{DM} \text{ kg}_N^{-1}$ ) but NUpE was similar between the two genotypes (Table 8) This resulted in 'Futura 75' having a higher NUE ( $58.4 \pm 2.6 \text{ kg}_{DM} \text{ kg}_N^{-1}$ ) than 'Fibror79' ( $50.0 \pm 1.8 \text{ kg}_{DM} \text{ kg}_N^{-1}$ ). The effect of nitrogen fertilisation was not significant on any of the three parameters.

In PC21, the effect of genotype was not significant on any of the three parameters, while both NUE and NUpE significantly decreased with increasing levels of nitrogen fertilisation (Table 8). No interaction effect between genotype and nitrogen fertilisation could be observed in any of

the three environments (Table 8).

## 4. Discussion

The aboveground biomass was in the range of values usually reported for 'Futura 75' and 'Fibror 79' in Italy and the British Isles (Struik et al., 2000; Cosentino et al., 2013; Finnan and Burke, 2013a, 2013b; Tang et al., 2016; Grégoire et al., 2021). Overall, a nitrogen response was not seen in PC21, whilst there was a clear response in PC20. In AB21, the effect of nitrogen was ambiguous: despite a trend for higher biomass at increasing levels of nitrogen fertilisation, particularly at seed maturity (Fig. 2), nitrogen did not have any significant effects on aboveground biomass. The total nitrogen taken up by the crop was very similar between AB21 and PC20 while it was far higher in PC21, suggesting that the relatively little effect of nitrogen fertilisation on aboveground biomass in AB21 in comparison to that observed in PC20 could be explained by specific environmental conditions such as e.g. the relatively low maximum PAR intensity in AB21 as reported by Eriksen and Whitney (1981) and Stagnari et al. (2015).

For both cultivars in AB21, although aboveground biomass was not significantly affected by nitrogen fertilisation, seeds and threshing residues were very responsive to it (Supplementary material S6). In hemp, the stems were usually reported to display a stronger response to nitrogen fertilisation than the seeds or flowers (Vera et al., 2004; Finnan and Burke, 2013b; Tang et al., 2017a), which differ from the results of the present work. The reasons for the bigger response to nitrogen of seeds and threshing residues over stems in this work remain however unclear.

Because the levels of soil mineral nitrogen before fertilisation were higher in PC20 and AB21 than in PC21, it is probable that differences of meteorological conditions and of soil characteristics (e.g. soil organic matter) across the three environments have led to a greater mineralisation and availability of nitrogen in PC21 than in the other two environments (Vagstad et al., 1997). This is consistent with the much higher NUpE which exceeded  $1.0 \text{ kg}_N \text{ kg}_N^{-1}$  in PC21, on average double than that in the other sites. These results demonstrate that nitrogen fertilisation requirements of hemp are highly dependent on soil fertility characteristics, in accordance with previous reports (Ehrensing, 1998; Amaducci et al., 2015; Fike, 2016).

Nitrate leaching is not expected to have had a strong impact on the experiment in AB21: despite the sandier soil and the more important rainfalls in AB21 than in Italy, nitrogen fertilisation was delayed to a moment when rainfalls were particularly low which coincided with a period of high nitrogen demand for hemp (Ivonyi et al., 1997). The delayed fertilisation is not thought to have significantly impacted this trial's results as Finnan and Burke (2013b) did not report significant effects of a delayed nitrogen fertilisation on biomass productivity between a fertilisation realised at sowing and a fertilisation realised 44 days after sowing. However, the fact that the fertilisation had to be delayed in AB21 emphasises the importance of limiting nitrogen losses in environments prone to abundant rainfalls, and split applications of nitrogen can be considered as a viable way to do so (Finnan and Burke, 2013b).

Overall, the two cultivars reached comparable levels of biomass production, in particular under non-limiting nitrogen conditions (PC21). This is consistent with other comparisons between yellow and green hemp genotypes of similar flowering date (Cosentino et al., 2013; Tang et al., 2016). Still, the slight but significant difference of productivity observed between the two cultivars in PC20 suggests that 'Fibror 79' is less productive than 'Futura 75' under nitrogen limiting conditions, but not under non-limiting nitrogen conditions as this difference was neither observed in PC21, nor in AB21. Because both cultivars displayed the same phenological development, the difference of productivity observed in PC20 can be attributed to a difference in the rate of biomass production, which can partially be explained by differences of LAI during the early growth (Fig. 3). The increased LAI in 'Futura 75' led to smaller

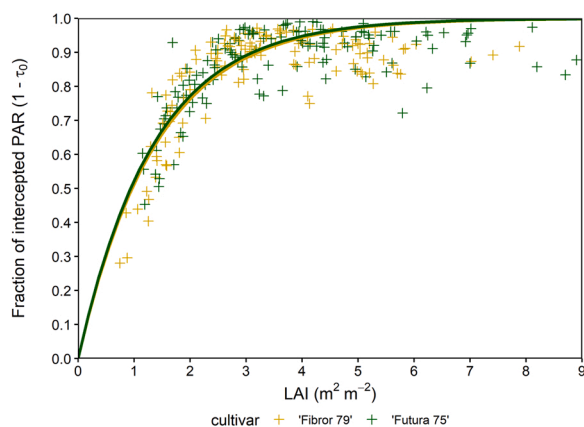


Fig. 4. Light extinction curves (solid lines, Eq. 3,  $n = 128$ ) fitted to all data points from PC20 or PC21 for 'Futura 75' and 'Fibror 79'.

**Table 7**

Leaf absorbance over the 400–700 nm range of the two cultivars in AB21, at S2, for each layer of the canopy; and significance of the effects of genotype, nitrogen, and of their interaction on the results obtained. Data presented are the mean  $\pm$  standard error ( $n = 16$ ).

Canopy layer (% of relative crop height)		Absorbance		Significance (p-value)		
		'Futura 75'	'Fibror 79'	Genotype	Nitrogen	Genotype $\times$ Nitrogen
100 – 90%		90.5 $\pm$ 0.4	88.3 $\pm$ 0.5	5.4 e <sup>-4</sup>	2.9 e <sup>-3</sup>	0.69
90 – 75%		90.4 $\pm$ 0.4	88.1 $\pm$ 0.5	2.2 e <sup>-3</sup>	0.19	0.70
75 – 60%		89.3 $\pm$ 0.5	85.3 $\pm$ 0.6	9.7 e <sup>-5</sup>	0.84	1.00
60 – 0%	upper	87.2 $\pm$ 0.6	81.4 $\pm$ 0.9	5.1 e <sup>-6</sup>	0.12	0.18
	Mid	77.9 $\pm$ 1.2	68.8 $\pm$ 1.8	1.4 e <sup>-4</sup>	0.49	0.07
	lower	53.3 $\pm$ 3.0	36.6 $\pm$ 2.0	1.4 e <sup>-7</sup>	6.0 e <sup>-3</sup>	4.4 e <sup>-5</sup>

amounts of light transmitted through the canopy compared with 'Fibror 79', and therefore to a potentially higher canopy assimilation rate in 'Futura 75'. Increased LAI, leading to increased light interception has been shown to be related to increased canopy carbon assimilation (Cock et al., 1979; Keating et al., 1982; Mandal and Sinha, 2004). In hemp, Meijer et al. (1995) showed that fast LAI growth early in the season increases total PAR intercepted by the crop, and thus is an important driver of productivity. However, later in the season (from S2 onward), although the difference of LAI remained significant between the two cultivars, there were no differences in light transmission. The saturation of canopy absorption by LAI greater than 2.5 m<sup>2</sup> m<sup>-2</sup> (Fig. 4), is similar to that reported by Meijer et al. (1995), but a higher LAI (over 6 m<sup>2</sup> m<sup>-2</sup>) was required to achieve saturation in a high solar radiation environment (Cosentino et al., 2013). This indicates that the observed difference of LAI between the two cultivars in PC20 might not have been the sole driver of the difference in biomass productivity, as the difference of biomass productivity attained at the end of the season between the two cultivars was not totally determined at S2 (average difference between the two cultivars of 0.97 t ha<sup>-1</sup> and 1.54 t ha<sup>-1</sup> at S2 and S6, respectively, in PC20).

'Fibror 79' appeared to be characterised by a lower rate of biomass production than 'Futura 75' under nitrogen-limiting conditions even when both cultivars transmit the same amount of PAR. This implies that 'Fibror 79' is either characterized by a lower radiation use efficiency (RUE) (de Wit et al., 1978; Lee et al., 2003) and/or by a lower leaf light absorption and faster senescence observed here, which could explain in return the slower LAI development in 'Fibror 79' than in 'Futura 75'. Leaf absorbance measured in AB21 indeed showed a difference of light absorption between the two cultivars but it did not result in significant differences of biomass productivity in this environment. Impollonia et al. (2022) showed that 'Fibror 79' was characterised by a lower leaf chlorophyll content than 'Futura 75' in PC20 and PC21 using leaf samples from the same experiment, which is consistent with the lower absorption found in AB21. By considering that total leaf nitrogen content was similar in PC20 and PC21, it can be hypothesised that the two cultivars are characterised by a difference in the balance between (i) the nitrogen allocated to the Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) which is of prime importance under high light at high photosynthetic rates, and (ii) the nitrogen used in light harvesting components and chlorophyll binding which must be maximised under low light conditions (Kumar et al., 2002). The existing difference of a gradient of light absorption across the relative crop height reported in this work could represent an interesting lever for action to select hemp cultivars adapted to different light environments (Walker et al., 2018; He et al., 2020).

This supposed difference of RUE between the two cultivars under nitrogen-limiting conditions cannot be explained by a higher NUpE of 'Futura 75' over that of 'Fibror 79'. Indeed, NUpE was never lower for 'Fibror 79' than for 'Futura 75', which contradicted the statements of Berenji et al. (2013) and Thouminot (2015) and failed to support the hypotheses of the present work. Instead, 'Futura 75' showed a significantly higher NUpE than 'Fibror 79' in PC20 and AB21, confirming a trend for 'Futura 75' to display a slightly higher efficiency of resource

utilisation, this difference between the two cultivars being dependant on nitrogen availability and in general, on the growth environment.

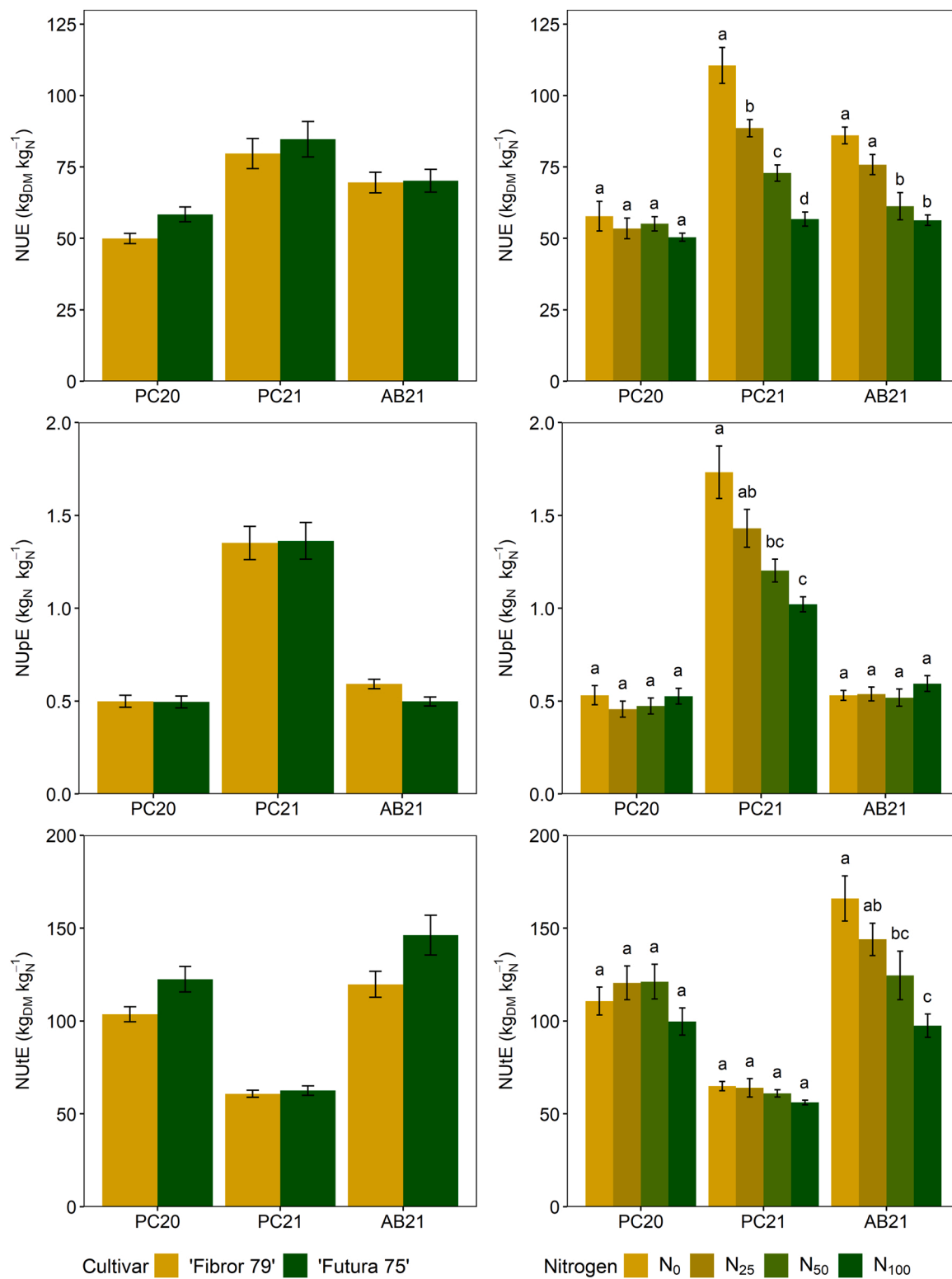
Overall, 'Futura 75' appeared to be better suited than 'Fibror 79' for dual- and multi-purposes production in Italy, as it produced significantly more threshing residues in PC20 and more seeds in both years than 'Fibror 79'. The maximum seed yield reported in Italian environments (0.47 t ha<sup>-1</sup> across all combinations of genotype  $\times$  nitrogen) was low when compared to previous data; for example, Legros et al. (2013) reported a mean seed yield of 1.0 t ha<sup>-1</sup> for different cultivars over a twelve-years period. In PC20 and PC21, a high fraction of seed was found to be empty in both years (in-field observation), which might be the consequence of water shortage in PC20 and of high temperatures during the seed filling period, as seed yield is particularly susceptible to these stresses (Campbell et al., 2019; Bahador and Tadayon, 2020; Baldini et al., 2020; Ferfuaia et al., 2021). Pests and predation by birds (in both years) and a gale that occurred two days before seed harvest in PC20 might also have contributed to the low seed yield.

In Wales instead, 'Fibror 79' produced more threshing residues and had the same seed productivity as 'Futura 75'. In this site, seed yield was particularly high (up to 2.23 t ha<sup>-1</sup>) compared to seed productivity in Italy. Similar differences of seed yields across four environments in Europe were reported by Tang et al. (2017a) with the cultivar 'Futura 75', but the highest seed yields were found in Piacenza, contrary to this work. In environments similar to AB21, 'Fibror 79' might be a better-suited cultivar because of its high production of threshing residues, seeds and fibre. 'Fibror 79' also appears to be a better cultivar for single-use fibre production, as it had significantly higher bast fibre yields than 'Futura 75' in all three environments.

It is impossible to conclude on the fact that the difference observed between the two cultivars are solely due to the genotype that drives the "yellowness", or if these differences were driven by other genetic variations between the two cultivars. Three main research axes could allow to better understand the implications of the yellow genotype in hemp: firstly, an assessment of the differences of fibre processability and quality between several yellow and green cultivars could be made, targeting the most relevant parameters for industrial applications to assess the fibre's commercial value. Secondly, a molecular investigation might be the most suited tool for identifying the genetic driver of the yellowness and it could allow to determine whether the yellow trait is linked to the high stem processability of YSCs or not. Thirdly, the study and modelling of gas exchanges under varying levels of nitrogen availability, at leaf and canopy scales, should provide a better understanding of the mechanisms underlying the differences of growth rate and light use efficiency between green and yellow genotypes. To do so, it could be possible to make use of the existence of a small percentage of plants displaying a green phenotype in the cultivar 'Fibror 79' (about 1% in the seed lots used in this work).

## 5. Conclusion

This work investigated the differences of yield and ecophysiology between a yellow hemp cultivar ('Fibror 79') and a green one ('Futura 75'), hypothesising that a low relative nitrogen uptake efficiency was



**Fig. 5.** Effect of genotype (left) and nitrogen fertilisation (right) on NUE (kg of biomass produced per kg of available soil nitrogen in the 0–60 cm layer), NUPE (kg of nitrogen taken up by the crop per kg of available soil nitrogen in the 0–60 cm layer) and NUtE (kg of biomass produced per kg of nitrogen taken up by the crop), in the three environments of study, measured at full-flowering. Bars and error bars represent the mean and standard error, respectively (n = 16 for genotype effect and n = 8 for nitrogen fertilisation effect). For each parameter and within each environment, different letters indicate significantly different conditions of nitrogen fertilisation after Tukey's HSD procedure ( $\alpha = 0.05$ ).

**Table 8**

p-values of the effects of genotype (G), nitrogen fertilisation (N) and of their interaction (G × N) on NUE, NUPE, and NUTE in each environment, assessed by two-way anova. Bold values highlight the significant effects ( $\alpha = 0.05$ ).

Parameter	Factor	PC20	PC21	AB21
NUE	G	<b>0.032</b>	0.22	0.86
	N	0.68	<b>1.2 e<sup>-8</sup></b>	<b>1.7 e<sup>-5</sup></b>
	G × N	0.98	0.63	0.79
NUPE	G	0.94	0.91	<b>0.017</b>
	N	0.61	<b>1.4 e<sup>-4</sup></b>	0.49
	G × N	0.77	0.13	0.88
NUTE	G	<b>0.021</b>	0.55	<b>0.010</b>
	N	0.19	0.17	<b>2.8 e<sup>-4</sup></b>
	G × N	0.41	0.092	0.62

the cause of the yellow trait. Results obtained failed to support this hypothesis as the Nitrogen Uptake Efficiency was not significantly different between the two cultivars. Instead, the Nitrogen Utilisation Efficiency appeared to be significantly lower in 'Fibror 79' than in 'Futura 75' at low levels of nitrogen availability, enabling 'Futura 75' to reach a slightly but significantly higher aboveground biomass than 'Fibror 79'. These differences were at least partially driven by differences of growth rate during the vegetative phase, potentially implying a lower rate of carbon assimilation and a lower light use efficiency of 'Fibror 79' over 'Futura 75' at low levels of nitrogen availability. This work also highlighted that the choice of cultivar for a given harvestable outputs depends on the environment, as 'Futura 75' is better suited for dual- and multi-purpose productions in Italy, while 'Fibror 79' appears better suited for dual- and multi-purpose productions in Wales. 'Fibror 79' is also better suited for single-use fibre production because of its higher level of bast fibre content and fibre yield.

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## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Stefano Amaducci reports financial support was provided by GRACE. Alan Gay reports financial support was provided by Smart Expertise program from Welsh government. co-author Stefano Amaducci is an associate editor of Industrial Crops and Products.

## Data Availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2023.116360.

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