Field Crops Research 121 (2011) 381–391

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Biomass production and nitrogen accumulation and remobilisation by *Miscanthus* × *giganteus* as influenced by nitrogen stocks in belowground organs

L. Strullu[a], S. Cadoux[a,*], M. Preudhomme[a], M-H. Jeuffroy[b], N. Beaudoin[c]

[a] *INRA, US1158 Agro-Impact, F-80200 Estrées-Mons, France*
[b] *INRA, UMR 211 Agronomie INRA AgroParisTech, EGER, F-78850 Thiverval-Grignon, France*
[c] *INRA, US1158 Agro-Impact, F-02000 Laon, France*

ARTICLE INFO

Article history:
Received 1 July 2010
Received in revised form 5 January 2011
Accepted 9 January 2011

Keywords:
Harvest date
Apparent nitrogen fluxes
Rhizome
Perennial reserves
Nitrogen fertilisation
Biomass crop

ABSTRACT

The nitrogen (N) requirement of dedicated crops for bioenergy production is a particularly significant issue, since N fertilisers are energy-intensive to make and have environmental impacts on the local level (NO_3 leaching) and global level (N_2O gas emissions). Nitrogen nutrition of *Miscanthus* × *giganteus* aboveground organs is assumed to be dependent on N stocks in belowground organs, but the precise quantities involved are unknown. A kinetic study was carried out on the effect of harvest date (early harvest in October or late harvest in February) and nitrogen fertilisation (0 or 120 kg N ha^{-1}) on aboveground and belowground biomass production and N accumulation in established crops. Apparent N fluxes within the crop and their variability were also studied.

Aboveground biomass varied between 24 and 28 t DM ha^{-1} in early harvest treatments, and between 19 and 21 t DM ha^{-1} in late harvest treatments. Nitrogen fertilisation had no effect on crop yield in late harvest treatments, but enhanced crop yield in early harvest treatments due to lower belowground biomass nitrogen content. Spring remobilisation, *i.e.* nitrogen flux from belowground to aboveground biomass, varied between 36 and 175 kg N ha^{-1}, due to the variability of initial belowground nitrogen stocks in the different treatments. Autumn remobilisation, *i.e.* nitrogen flux from aboveground to belowground organs, varied between 107 and 145 kg N ha^{-1} in late harvest treatments, and between 39 and 93 kg N ha^{-1} in early harvest treatments. Autumn remobilisation for a given harvest date was linked to aboveground nitrogen accumulation in the different treatments. Nitrogen accumulation in aboveground biomass was shown to be dependent firstly on initial belowground biomass nitrogen stocks and secondly on nitrogen uptake by the whole crop.

The study demonstrated the key role of belowground nitrogen stocks on aboveground biomass nitrogen requirements. Early harvest depletes belowground nitrogen stocks and thus increases the need for nitrogen fertiliser.

1. Introduction

The use of dedicated crops for production of biofuels to replace fossil fuels is one way to reduce anthropogenic greenhouse gas emissions (Smith et al., 2000). *Miscanthus* × *giganteus* is a perennial rhizomatous grass employing the C4 photosynthetic pathway, which originates from Asia and was introduced into Europe in the 1930s. It has been described as having high potential biomass production with a low nitrogen requirement (Lewandowski et al., 2000). These traits are likely to lead to significant energy production per hectare and high reductions in greenhouse gas emissions when used for fossil fuel substitution (Clifton-Brown et al., 2007; Heaton et al., 2008). The nitrogen (N) requirement is a particularly significant issue, because N fertilisers are energy-intensive to manufacture and so greatly affect the energetic balance of crops (Boehmel et al., 2006). Moreover, losses following N fertilisation have environmental impacts on the local level (e.g. NO_3 leaching) and the global level (e.g. N_2O gaseous emissions). Biomass production by *M. giganteus* has been described as being dependent on soil water availability, air temperature and precipitation (Richter et al., 2008), but there is no consensus yet in terms of this crop's nitrogen fertilisation requirement. Indeed, many authors suggest that N fertilisation has no effect on biomass production (Christian et al., 2008; Clifton-Brown et al., 2007; Danalatos et al., 2007; Himken et al., 1997) whereas others report that nitrogen fertilisation is needed to achieve maximum biomass production (Boehmel et al., 2006; Cosentino et al., 2007; Ercoli et al., 1999). However, a consensus view is that the nitrogen requirement of *M. giganteus* to achieve maximum biomass yields is low compared with that of other crops (Lewandowski and Schmidt, 2006). This is mainly due

* Corresponding author. Tel.: +33 322857515; fax: +33 22856996.
E-mail address: stephane.cadoux@mons.inra.fr (S. Cadoux).

0378-4290/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.fcr.2011.01.005

to N cycling within the crop. In spring, part of the rhizome nitrogen stocks are remobilised from belowground to aboveground organs (hereafter referred to as spring remobilisation). Part of the nitrogen accumulated in aboveground parts is subsequently remobilised from aboveground to belowground organs (hereafter referred to as autumn remobilisation) during autumn and winter (Beale and Long, 1997; Christian et al., 2006; Himken et al., 1997). However, the exact crop requirements in terms of N fertilisation have not been defined. Few studies have taken into account the contribution of the rhizome in the nitrogen nutrition of the crop, and the factors that affect spring and autumn remobilisation are not known. In fact, the amounts of remobilised nitrogen in spring and autumn reported by Beale and Long (1997) and Himken et al. (1997) differ, possibly owing to differences in belowground biomass nitrogen stocks, aboveground biomass nitrogen accumulation and climate conditions.

M. giganteus is currently used in combustion to produce heat and electricity, and is thus harvested in late winter to benefit from improved quality with regard to combustion processes, *i.e.* low mineral and moisture content (Lewandowski and Heinz, 2003). The development of an industrial process for converting cellulose to ethanol is likely to make early harvest of green material interesting, since the quality criteria for this type of conversion relate to lignocellulose content and recalcitrance (Karp and Shield, 2008). In a recent study, Le Ngoc Huyen et al. (2010) showed that saccharification yields of early harvested biomass were higher than those of late harvested plants. However, early harvest could increase the crop nitrogen requirement due to preventing or limiting leaf losses and autumn remobilisation, which in turn could prevent or limit nitrogen recycling in the soil–crop system.

The aims of this study were to determine: (i) the impact of harvest date and nitrogen fertilisation on aboveground and belowground biomass production, (ii) the impact of harvest date and nitrogen fertilisation on aboveground and belowground nitrogen accumulation, and (iii) the contribution and determinants of nitrogen cycling on nitrogen accumulation in *M. giganteus*.

2. Materials and methods

2.1. Experimental site and trial setup

The experimental site is located in the Picardie region of Northern France (49°52′N, 3°00′E). The soil is a deep silt loam (Ortic luvisol) and is characterised by pH 7.6, 19% clay, 74% silt and 5% sand. The climate is oceanic, with mean rainfall of 625 mm per year and mean temperature of 10.7 °C for the past 10 years. *Miscanthus* × *giganteus* was planted in May 2006 at a density of 15,625 plants ha^{-1} in a randomised block design. The previous crop was wheat, harvested in July 2005. After planting in 2006, two applications of herbicide were necessary to control weeds but no fertiliser was applied. The density after the first season of growth was 14,941 plants ha^{-1}. During the second year (2007), four different treatments with three replicates were established. Treatments varied in terms of nitrogen (N) fertiliser rate: 0 kg N ha^{-1} (N0) or 120 kg N ha^{-1} (N1), and harvest date: early harvest (E) or late harvest (L). The whole plots were harvested in October for early harvest and in February for late harvest. Plot size was 360 m^2 (12 m × 30 m), with 540 plants per plot. Each year, from 2007, nitrogen was applied as ammonium nitrate in late April. The soil mineral nitrogen (SMN) content was determined in March, before N fertilisation. In each plot, six soil cores were divided into five layers of 30 cm thickness. The six soil cores for each layer were pooled before N analysis. The temperature measured during the two years of growth was comparable to the 10-year average. The weather was drier in 2009 than in 2008. It was wetter than average in 2008 and drier in 2009 (Fig. 1).

2.2. Biomass sampling

In each of the four treatments, aboveground biomass production was estimated on six occasions in 2008 (third year of growth) and 2009 (fourth year of growth) and belowground biomass production on five and six occasions in the third and fourth years of growth, respectively. Six adjacent plants were harvested to measure the aboveground biomass on each occasion. The number of stems per plant was determined, and then a subsample was used for estimation of the moisture content. The stems (S), green leaves (GL) and dead leaves (DL) were separated from a second subsample, in order to estimate the proportion of each organ. The first and second subsamples were dried for four days at 65 °C and then weighed again in order to determine dry matter weight. In order to take better account of canopy variability, the number of stems per plant was counted in an undisturbed area (hereafter referred to as area *A*) of 25 m^2 (40 plants) in all blocks, to determine the number of stems per hectare (NS). A nylon net (mesh size 1 cm × 1 cm) was placed on the soil surface before leaf abscission in order to collect abscised leaves during senescence from six plants per block (corresponding to a 3.84 m^2 area) in late harvest treatments. Abscised leaves that had fallen to the ground were collected regularly (every two weeks) and analysed to quantify the nitrogen lost during winter by this process. The leaves were dried for four days at 65 °C, and then nitrogen concentration was determined (Section 2.3). The aboveground biomass at each harvest was calculated as:

$$W_A = \frac{dm_A}{ns} NS \quad (1)$$

where W_A is the aboveground biomass production (t DM ha^{-1}), dm_A the aboveground dry matter of the six plants (kg), ns the number of stems of the six plants and NS the number of stems per hectare determined in area *A*.

The dry weight per hectare of stems (W_S), green leaves (W_{GL}) and dead leaves (W_{DL}) was then determined by multiplying the aboveground biomass production (t DM ha^{-1}) by the proportion of each respective organ (%).

According to Midorikawa et al. (1975), there is a linear relationship between aboveground biomass of a *Miscanthus sinensis* plant and its rhizome biomass. We also observed a linear relationship between aboveground and belowground biomass, but this relationship varied as a function of sampling date (data not shown). Therefore, in order to determine the belowground biomass, we extracted the rhizome of one plant, the closest to the median among the six harvested plants. The median plant was determined for each block of each treatment on a number of stems per plant basis, after counting the stems of each plant in area A. On each sampling occasion except May 2008, the rhizome and associated roots were extracted at a depth of 25 cm. After extraction, belowground biomass was washed and divided into rhizome (Rh) and roots (Ro). All organs were dried for four days at 65 °C until constant weight and then weighed in order to determine their dry matter weight. Belowground biomass was calculated as:

$$W_B = (dm_{Rh} + dm_{Ro})NP \quad (2)$$

where W_B is the belowground biomass (t DM ha^{-1}), dm_{Rh} the rhizome biomass (t DM ha^{-1}), dm_{Ro} the root biomass (t DM ha^{-1}) and NP the number of plants per hectare determined in area *A*.

Unfortunately, the median plant had not been determined before harvest in October 2007 and belowground biomass in February 2008 in early harvest treatments was not sampled according to this protocol, so these data were removed from the analysis to avoid random variability.

The cumulated degree-days (CDD) during each year of growth were calculated on a 6 °C basis from emergence, as suggested by