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The significance of litter loss and root growth on nitrogen efficiency in normal and semi-dwarf winter oilseed rape genotypes

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ABSTRACT

Winter oilseed rape (*Brassica napus* L.) has a high capacity for nitrogen (N) uptake, but still leaves substantial amounts of N in the soil after harvest, partly due to the N lost through shed leaves. Reduced leaf litter losses or increased N uptake might therefore be possible ways of increasing the N efficiency of winter oilseed rape. In order to determine genotype differences in litter loss and root growth, we examined biomass production, leaf litter loss, N content, yield, root distribution and root depth of winter oilseed rape, as well as soil N content, in a two-year field experiment conducted on sandy loam soils. Three genotypes were studied: a semi-dwarf (SD) hybrid and two normal hybrids. All genotypes were treated with low and high N fertilization levels of 120 and 280 kg N ha^{-1}, respectively. Root growth was studied by means of minirhizotrons to 2.3 m soil depth. Lost leaves contributed significantly to the N surplus left in the soil after oilseed rape. Estimations based on litter loss measurements revealed that more than 100 kg N ha^{-1} potentially could be lost during the growing season. Generally, estimated total litter loss and accumulated N loss did not differ between the studied genotypes, whereas more N was lost from the high N treatments. In spring, the nitrate (NO_3^-) levels throughout the soil profile were low, indicating the efficiency of oilseed rape in taking up available NO_3^- and reducing the risk of leaching losses during autumn and winter. The SD genotype was not found to have less leaf biomass, but had lower yields leading to lower N use efficiency (NUE) as well as lower N uptake efficiency (NUpE). Thus, the hypothesis that SD would have less biomass and consequently reduced litter loss leading to increased N efficiency was rejected. Generally, SD took up less N and tended to leave more NO_3^- in the upper soil layers at harvest. At maturity, roots reached at least 2.3 m depth, with no significant genotype differences in depth and intensity. This implied that genotype differences in N efficiency and yield was not related to root growth differences, indicating that root growth is not the most obvious target in order to improve N efficiency of winter oilseed rape. Improving remobilization of N from the large amount of biomass lost during the growing season or decreasing the amounts of biomass produced and lost might be more obvious and effective targets.

1. Introduction

Increasing nitrogen (N) efficiency of common crops is a prerequisite to meet the future demands for food, feed and fuel in a sustainable way. Oilseed rape (*Brassica napus* L.) is the third largest source of vegetable oil in the world and the most widely grown oil plant in Europe (USDA, 2015). It is a crop with a high capacity for uptake of mineralized N from the soil (Laine et al., 1993), and winter oilseed rape is therefore considered a good catch crop reducing nitrate leaching during autumn and winter. Although oilseed rape has a high N uptake during the growth season, it leaves substantial amounts of N in the soil after harvest (Rathke et al., 2006), leading to increased risk of nitrate leaching during the autumn. The high level of N residues in the soil is one of the reasons that oilseed rape is a good pre-crop for cereals, while another reason is the break-crop function, as it disrupts the life cycle of soil-borne pathogens (Kirkegaard et al., 1997). However, the high amounts of soil N left after harvest implies that the N efficiency of oilseed rape could be increased.

Improvement of N efficiency of winter oilseed rape can be approached in several ways; either by improving the N uptake from the soil (Garnett et al., 2009; Ulas et al., 2012), by improving the ability of the crop to hold on to this N through remobilization (Masclaux-Daubresse et al., 2010; Ulas et al., 2013), or by reducing the aboveground biomass and thereby the amount of N lost through litter losses (Sieling and Kage, 2008).

Increased N uptake from the soil could be reached e.g. by improving the actual uptake mechanisms from soil to root (Good

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et al., 2004), or by breeding for root architecture with more branched or deeper rooting, which would bring the roots into contact with more soil N (Garnett et al., 2009). As oilseed rape has a deep and well-distributed root system (Barraclough, 1989), the potential for further gain by improving rooting depth and distribution may be limited. However, in a field study of two genotypes differing in N efficiency, the N efficient genotype was shown to invest more in root growth in the top soil layers during the vegetative phase, with increased total root length (Ulas et al., 2012).

N uptake in winter oilseed rape occurs mainly in autumn and again in spring from stem elongation to flowering, whereas little N uptake occurs during pod development and ripening (Malagoli et al., 2004). Consequently, most of the pod N derives from remobilized N previously accumulated in leaves and stem (Malagoli et al., 2005). Leaves from winter oilseed rape are lost continuously during the growing season. From late autumn to early spring the first set of leaves are lost, while the second set of leaves, produced in the early spring, are mainly lost from flowering until maturity. Increasing the ability to efficiently remobilize N from the leaves before shedding would be a possible way to increase the N efficiency. Zhang et al. (2010) showed that highly N efficient oilseed rape genotypes have a greater speed of N remobilization, a larger amount of N remobilization, more N remobilized into the seed and less N remobilized into the pod husk. N remobilization is generally thought to be sink-limited for plants with a high N content and source-limited for plants with a low N content (Gombert et al., 2010). However, in a field study Ulas et al. (2013) found that the genotypic variation of N remobilization efficiency was lower than the genotypic variation of N uptake and distribution in the plant. N fertilization levels were not found to affect the relative N remobilization (Ulas et al., 2013).

Finally, reducing the amount of leaf biomass, and by that lost biomass, could also be a way of increasing N efficiency. Reducing the leaf losses would lead to less N lost and thereby to more efficient N use. Determining the litter loss of oilseed rape is a time consuming and difficult task, and therefore studies on this are limited. Still, it has been found that a substantial amount of N is lost with leaf litter; up to 45 kg N ha^{-1} year^{-1} when determined from stem elongation to maturity (Dejoux et al., 2000; Malagoli et al., 2005). Losses of approximately 100 kg N ha^{-1} year^{-1} have been estimated when including lost leaves before stem elongation, lost due to winter frosts where green leaves with high N content are lost (Dejoux et al., 2000).

When the lost leaves decompose, the mineralized N may be available for the crop again, depending on the time of year when the leaves are lost as well as the C/N ratio of the leaves. Hence, if plants effectively remobilize the N from the leaves before they are lost, the resulting high C/N ratio may lead to net immobilization of N during leaf decomposition and reduce the subsequent amount of soil N.

Leaf litter turnover and root distribution patterns are both considered important for increased N efficiency in oilseed rape (Malagoli et al., 2005; Ulas et al., 2012), but these factors are not studied to the same degree as N remobilization patterns. In order to determine genotype differences in N efficiency, we examined leaf litter loss, N content, root distribution and root depth in three winter oilseed rape genotypes; a semi-dwarf and two normal hybrids. It was hypothesized that (1) genotypic differences occur in litter loss of winter oilseed rape, that the litter loss is dependent on N fertilization and that the amount of litter loss affect the N efficiency of the genotype, (2) the semi-dwarf genotype has less vegetative biomass and thus a reduced litter loss compared to the normal hybrids leading to increased N efficiency, and (3) the rooting depth as well as root distribution are dependent on N fertilization and less dependent on genotype.

Table 1
Clay, silt, sand and chemical composition of the soil down to 2.3 m. Values are averaged for the experimental fields.

Depth	Clay (%)	Silt (%)	Sand (%)	C (%)	pH	P (mg kg^{-1})	K (mg kg^{-1})
0–0.5 m	15.5	13.8	69	1	6.6	26.8	117.5
0.5–1 m	20.3	14.8	64.5	0.5	6.9	15.3	59.8
1–2.3 m	19	17.9	62.3	0.2	7.5	6.2	50.7

2. Materials and methods

2.1. Field site and experimental design

The experiment was carried out from August to July in 2011–2012 (exp. 1) and 2012–2013 (exp. 2) in two separate fields at the University of Copenhagen, Department of Plant and Environmental Sciences, in Taastrup, Denmark (55°40′N; 12°18′E), on a sandy loam soil (Agrudalf) (Table 1).

Temperature data (Fig. 1) was obtained from a meteorological station located less than 700 m from the experimental fields, and precipitation data was obtained from two different meteorological stations located less than 15 km from the experimental fields. Accumulated precipitation was 611 mm from 1 August 2011 to 31 July 2012, and 459 mm from 1 August 2012 to 31 July 2013. Accumulated average daily temperature (°C day) was calculated assuming a base temperature of 0 °C.

A DH population was derived from the cross of a dwarf isogenic line and a normal-type resynthesized rapeseed line (‘Alesi-*bzh* × H30’) (Foisset et al., 1995; Girke et al., 2011). The DH lines segregated into dwarf and normal types. These were crossed with a normal-type, male-sterile tester and resulted in both semi-dwarf and normal types. Three genotypes of these winter oilseed rape hybrids were grown; a semi-dwarf hybrid (SD) and two genotypes of normal hybrids, one (LB) shown to have low total biomass but high harvest index (HI) and yield in previous tests and one (HB) shown to have higher biomass but lower HI and yield in previous tests.

Two different N-fertilization treatments were applied; 120 (N120) and 280 (N280) kg N ha^{-1}. The genotypes were sown on 23 August 2011 (exp. 1) and on 20 August 2012 (exp. 2) with 3 kg seed ha^{-1}. The experiment was placed in a randomized complete block design with three replicates and with a plot size of 3 × 10 m. In exp. 1, the pre-crop was oat (*Avena sativa* L.), whereas winter barley (*Hordeum vulgare* L.) was pre-crop in exp. 2. Straw from the previous crop was removed from the field before ploughing. N fertilization was applied as inorganic N fertilizer (50% NO_3^-, 50% NH_4^+). During autumn in exp. 160 kg N ha^{-1} was applied in both N treatments; 30 kg N ha^{-1} on 16 September and, due to the poor growth, additionally 30 kg N ha^{-1} on 20 October. In exp. 2, 30 kg N ha^{-1} was applied on 18 September. Spring fertilization was divided in two. In exp. 1, 110 kg N ha^{-1} was applied to the high N treatments and 30 kg N ha^{-1} to the low N treatments on 18 March and again on 4 April. In exp. 2, fertilization was given later due to late frosts; 125 kg N ha^{-1} and 45 kg N ha^{-1} were applied to the high and low N treatments, respectively, on 14 April and again on 29 April. Base dressings of phosphorus and potassium were applied in spring as well. Herbicide was applied in autumn both years to control weeds, and pest insects were controlled by insecticides in autumn of exp. 1 and in spring of exp. 2.

2.2. Determination of litter loss, plant biomass and yield

Litter loss was determined during six two-week periods through the growing season: in late December/early January (BBCH 19), early March (BBCH 30–32), early April (BBCH 55–57), early May (BBCH 65), early June (BBCH 71–75), and late June/early July (BBCH