Leaf CO_2 assimilation and leaf dynamics in catch crops during autumn and winter at two levels of nitrogen supply

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Abstract

This study relates the leaf CO_2 assimilation and leaf dynamics of nitrogen catch crops to environmental conditions. Winter rye (*Secale cereale* L.) and fodder radish (*Raphanus sativus* L.) were grown as catch crops in an outdoor pot experiment at two rates of nitrogen supply (N2 higher than N1) in Wageningen, the Netherlands, from August 1993 until April 1994.

Biomass increased with increasing N supply. There was no net growth after mid-November. The number of tillers in rye and of appeared leaves in radish were higher in N2 than in N1. Leaf appearance rate increased with temperature in fodder radish and rye. Tillering in rye ceased in mid-October. Leaf lifespan was related to the temperature sum between leaf emergence and leaf death. Leaf lifespan was 478 ± 68 °C day in fodder radish. In rye, the leaf lifespan gradually decreased from 592 ± 66 to 389 ± 25 °C day and from 545 (1 observation) to 401 ± 64 °C day in N1 and N2, respectively. In young leaves, A_{max} (light-saturated CO_2 assimilation rate) was approximately 1.2 mg CO_2 m^{-2} leaf s^{-1} in September and 0.5 mg CO_2 m^{-2} leaf s^{-1} later on, independent of species and N supply. N supply affected the organic N concentrations only in the older leaves. A_{max} was not dependent on temperature at measurement (range: 12–19 °C in September, 5–15 °C from November until March), but, in contrast, strongly related to temperature and irradiance during the preceding growth period. Leaf nitrate concentrations increased with N supply. Water-soluble carbohydrate concentrations were higher in N1 than in N2 and higher in rye than in fodder radish. They fluctuated during the season. It is concluded that overall growth rates were limited by process rates other than that of leaf CO_2 assimilation per unit leaf area in both N1 and N2.

Keywords: autumn, CO_2 assimilation, catch crop, fodder radish, irradiance, leaf appearance, leaf lifespan, site filling, temperature, winter rye, winter

Introduction

Many growth processes in plants have been well quantified under controlled temperature and irradiance conditions. In the field, crops often react differently to environmental factors from plants under controlled conditions, and growth processes are

less well-understood in the field (Grace, 1988; Pollock & Eagles, 1988). Large variations in crop growth rates in the field in autumn and winter, at low irradiance and low temperatures (Vos, 1992; Lainé *et al.*, 1993), and the biomass production of overwintering crops, like nitrogen catch crops, cover crops, green manures, winter cereals, or grassland are only partly understood.

The effects of temperature, irradiance level and nitrogen supply on CO_2 assimilation rates have been studied under controlled conditions (e.g., Huner *et al.*, 1986; Dijkstra, 1989), but few data could be found for fluctuating autumn and winter field conditions. In some winter annuals, the optimum temperature for CO_2 assimilation shifts from 25–30 °C in summer to 15 °C in winter, while the maximum CO_2 assimilation rates per unit leaf area in winter are hardly lower than those in summer (Regehr & Bazzaz, 1976). In winter wheat grown under winter conditions (5–7 °C), however, the maximum CO_2 assimilation rate is about half that of plants grown at higher temperatures (25 °C) in a growth chamber (Sawada & Miyachi, 1974). In plants that were acclimated to winter conditions, light-saturated assimilation rates at air temperatures of 0 °C are about half those at the optimum temperature. The seasonal change in assimilation rates varies with species (Sawada & Miyachi, 1974).

At the molecular level, the tertiary and quaternary structure of ribulose bisphosphate carboxylase-oxygenase (RuBPCase) is influenced by growth temperature, resulting in a higher maximum carboxylation rate at low temperature and a lower temperature optimum in cold-hardened plants than in to non-hardened plants. Furthermore, thylakoids are altered by growth at low temperature, resulting in a greater capacity for electron transport (Huner, 1985). The combination of these features results in a higher maximum leaf CO_2 assimilation rate at low temperature in cold-hardened plants compared to non-hardened plants (Farquhar *et al.*, 1980). Irradiance conditions affect several factors determining the maximum leaf CO_2 assimilation: both the concentration of RuBPCase, and the capacity for electron transport are reduced at low irradiance levels, and mesophyll resistance is increased (Jones, 1992). But the effect of these changes on maximum leaf CO_2 assimilation in summer and winter in the field has not been well quantified.

Leaf appearance and turnover are important for determining the leaf area and thus for the estimation of gross and net growth in analysis of field experiments. Leaf appearance has often been related to temperature over winter (e.g. Hunt & Chapleau, 1986; Triboi-Blondel, 1988), but the variation is large. Leaf longevity data are scarce. In a perennial ryegrass (*Lolium perenne* L.) sward, the appearance and death rates of leaves are balanced, so that a constant number of leaves is present in the canopy (Harper, 1989). Therefore, leaf lifespan might also be related to temperature.

The aim of this study was to analyse growth and to determine rates of leaf CO_2 assimilation and leaf dynamics of catch crops during autumn and winter under Dutch conditions, and to relate process rates to the prevailing temperature and irradiance conditions. As catch crops are generally unfertilized and meant to minimize N losses from the soil, the processes were studied at two levels of N availability. Two contrasting species, chosen because they perform well as nitrogen catch crops, were compared. One was winter rye (*Secale cereale* L.), a monocot, which is frost resis-

tant and performs relatively well when sown late. The other was fodder radish (*Raphanus sativus* L.), a dicot of the Brassicaceae, which accumulates large amounts of nitrogen under Dutch autumn conditions but is not winter-hardy.

Materials and methods

Plant cultivation

Winter rye (cv. Halo) and fodder radish (cv. Pegletta) were sown in drained 7.6 l pots filled with coarse sand, and thinned to 9 plants per pot after emergence. Rye was sown on 17 August 1993, fodder radish on 25 August 1993. A crop canopy with 100 plants m^{-2} was approximated with the pots, both for rye and radish. To reduce border effects, the pots were surrounded by an extra row of pots with plants of rye and radish, respectively. For exposure to 'field' weather conditions, the plants were grown outdoors in Wageningen (51° 58' N), the Netherlands, until early April 1994. To reduce the risk of frost damage or early death, fodder radish was placed in an unheated greenhouse during frost periods from 19 to 20 October and from 16 November until 6 December. Despite this, the older leaves of fodder radish were affected by frost damage during the latter frost period.

There were two levels of nitrogen supply (Figure 1). One was aimed to be sufficiently high as not to limit crop growth (N2). N1 contained 30% of the N and 60% of the other nutrients of N2, so that growth was only limited by N shortage. Nutrients were added regularly using Steiner nutrient solution (Steiner, 1984). The N supply was matched to expected crop growth and nitrogen demand, following the patterns of irradiance and temperature (Figure 2). The soluble N content of the coarse sand was 57 ± 15 mg per pot (0.63 ± 0.17 g m^{-2}) before fertilization.
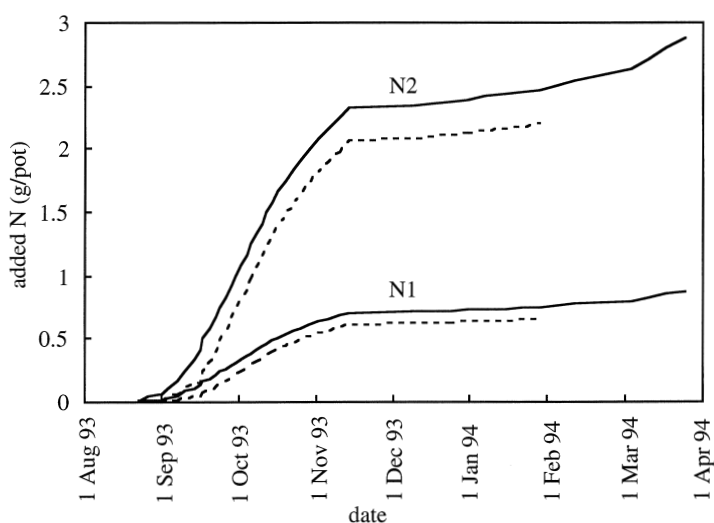

Figure 1. Cumulative amounts of N applied per pot during the experiment for fodder radish and winter rye at two N fertilisation levels. Solid line: rye; dashed line: radish.
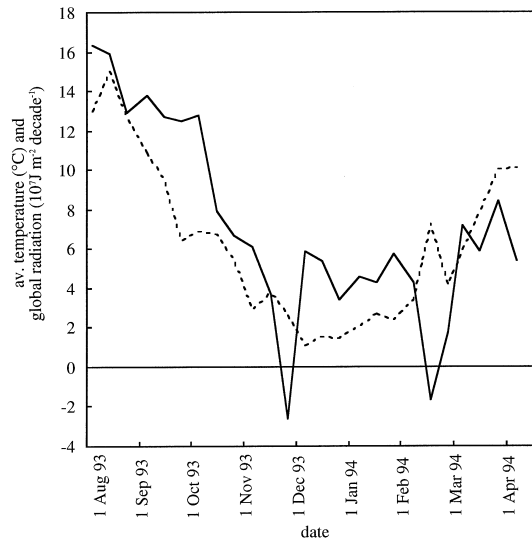
Figure 2. Average temperature and global irradiance per decade from August 1993 until March 1994. Solid line: temperature; dashed line: global irradiance.

Leaf dynamics

The appearance and death of leaves on the main stem in both species and the number of tillers in rye were recorded at intervals varying from two days to one month, dependent on the season (Table 1), so that generally not more than one leaf had appeared and not more than one or two leaves had died between successive leaf countings. A leaf was recorded as appeared when more than about 0.25 cm^2 of the new leaf was visible. A leaf was recorded as dead when more than 50% of its area was yellow, brown or frozen. Leaf lifespan was calculated for those leaves of which both appearance and death were timely recorded.

CO_2 assimilation and leaf analysis

At several moments during the experiment (Table 1) leaves from successive leaf positions on one stem or tiller were selected. Only leaves without any sign of frost damage were used. Young, full-grown leaves (age 1) were chosen because they were expected to have the highest assimilation rates. After September, also the second youngest and, when present, third youngest leaves on the same stem or tiller (age 2 and age 3) were selected. Light saturated net CO_2 uptake rates (A_{max}) and leaf chamber temperatures were measured with a portable leaf CO_2 assimilation meter (ADC Ltd., Hoddesdon, Herts. UK) on four leaves per age class per pot. A saturating light intensity was imposed with a Philips 12 V 75 W halogen lamp. The leaf temperature in the leaf chamber fluctuated during the days of measurement, due to warming by the lamp. To relate rates of CO_2 assimilation and overall growth to leaf characteristics, the leaves were harvested per age class per pot for further analysis after the A_{max} measurements.

Intact leaves were sampled for rye at all measurements, but for fodder radish only

Table 1. Dates of measurement of numbers of tillers, and appeared and dead leaves, biomass, A$_{max}$ and leaf composition. Days are marked with 'wr' or 'fr', if measurements were done only on winter rye or fodder radish, respectively. * On this date only young leaves of age 1 were measured, not of age 2 and 3.

month	leaf number	biomass number of tillers	A$_{max}$ leaf composition
August	30 wr		
September	1 wr, 3 wr, 6 wr, 8 wr, 10, 13, 15, 18, 21, 23, 27	27	24*
October	4, 11, 18, 25		
November	8	22	18–19
December	9		
January	10	26 fr	24 fr
February	7	10 wr	10 wr
March	4 wr	29 wr	29 wr

in September. After September, the thick veins in the fodder radish leaves were excluded, to make the samples representative for the leaf blades on which A$_{max}$ was measured. Leaf areas and dry weights and total N concentrations (Novozamsky *et al.*, 1974, 1983) were determined. Concentrations of nitrate (Walinga *et al.*, 1989) and water soluble carbohydrates (total reducing sugars; Bran & Luebbe method NL 208-89FT) were measured after extraction in water (Walinga *et al.*, 1989, but with heating of the samples in the extraction fluid to 100 °C for 15 minutes before filtering). Organic N concentrations were calculated as the difference between total N and nitrate concentrations.

Biomass

Dry weights were measured on samples of above-ground biomass of 3 or 4 plants from the pots used for the CO$_2$ assimilation measurements on the dates indicated in Table 1.

Use of weather data

Weather data were recorded at 2 km distance from the experimental site at the same elevation. Daily average temperature was calculated as the average of maximum and minimum temperature. Photoperiod was calculated according to Goudriaan & Van Laar (1994) for relating CO$_2$ assimilation rate to radiation conditions. To establish a relation between leaf lifespan and weather conditions, the leaf lifespan was related to temperature sum, with an assumed base temperature of 0 °C. For other species temperature sum proved a good measure for predicting leaf lifespan (Leong and Ong, 1983; Ingram, 1980). It is useful for modelling leaf turnover (e.g. Van Keulen & Seligman, 1987; Spitters et al., 1989).

To find the relation between temperature and leaf appearance rate, the average leaf appearance rate on the main stem between measurements was plotted against the average above-zero temperature in the same interval. Temperatures below zero were counted as zero, because leaf appearance rate was assumed to be zero at below zero temperatures (e.g. Hunt & Chapleau, 1986). Including below zero temperatures otherwise in the averages would yield an irrealistic view on the relationship above the base temperature. To reduce scatter due to measurement errors, intervals were chosen in which at least one leaf had appeared.

Statistical analysis

CO_2 assimilation measurements and leaf analysis were performed on 4 leaves per age class in each of 3 replicate pots in each combination of species, nitrogen supply and measurement date. Leaf dynamics and number of tillers (rye only) were recorded on 2 plants in 3 replicate pots in each combination of species and nitrogen supply. The pots were randomized per combination of species and N supply.

The experiment was set up as a split-plot design with species at the block stratum, N supply level at a sub-block stratum, month of measurement at a pot stratum and leaf age at the unit stratum. For biomass, A_{max}, and the concentrations of leaf organic nitrogen, nitrate and water-soluble carbohydrates, variance components were estimated by the method of residual maximum likelyhood (REML, Anonymous, 1993) and treatment effects were tested with the χ-squared test. No distinction could be made between the effects of block and of species. The block effect was assumed to be absent, which is plausible because soil and weather conditions were the same.

For testing a possible relation of A_{max} to leaf chamber temperature, a regression model was used with linear terms for the treatments and two-way interactions between the treatments and leaf chamber temperature.

The temperature sums during the lifespan of the leaves of each species were linearly fitted to leaf number. The effect of N supply level on the fitted lines was tested with the F-test. Time courses of the numbers of appeared and dead leaves were fitted per species with expolinear curves and those of the numbers of tillers in rye with second-order rational functions. With the help of these curves, the effect of N supply on the time course of tiller numbers and numbers of appeared and dead leaves was tested by the F-test. Moreover, the relation of leaf appearance rate to temperature, often found in literature (e.g., Hunt & Chapleau, 1986; Hay & Abbas Al-Ani, 1983), was tested by fitting it to temperature and temperature squared. Significance of the terms was tested with the F-test. All analyses were conducted in Genstat (Anonymous, 1993).

Results

Biomass & development

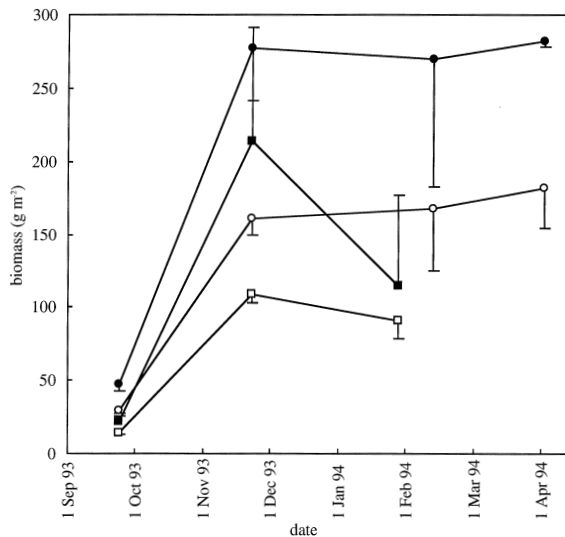Aboveground biomass (Figure 3) was higher in N2 than in N1 ($P < 0.001$) and did

Figure 3. Aboveground biomass in fodder radish and winter rye at N1 and N2 from August 1993 until March 1994. Standard deviations are given by bars in one direction, when larger than the symbol. Symbols: ─○─ = rye N1, ─●─ = rye N2, ─□─ = radish N1, ─■─ = radish N2.

not increase after the second measurement on 22 November. Net growth is also absent in field experiments, after mid-November (Vos & Van Der Putten, 1997). Apparently, under Dutch conditions most of the growth of catch crops takes place before mid-November. This is also to be expected because of the low levels of irradiance and temperature after early November (Figure 2). Biomass was higher in rye than in fodder radish, that was sown eight days later. The decrease in fodder radish biomass after November is attributed to normal turnover (cf. Vos & Van Der Putten, 1997) and to frost damage, which occurred despite transfer of the plants to the (unheated) greenhouse. Fodder radish froze to death in the second half of February, so that no later measurements could be made.

Stem elongation in fodder radish started around mid-October. Branches were initiated in leaf axils, but did not expand. Flower buds appeared from January onwards, but no flowering occurred. During the whole experiment, rye stems did not elongate.

Leaf and tiller dynamics

The numbers of appeared and dead leaves on the main stem are presented in Figure 4. Leaf appearance rate was high in September, and slowed down at about mid-October. The number of appeared leaves was higher in N2 than in N1 ($P < 0.001$ in fodder radish, $P = 0.007$ in rye), but in rye the difference was small and occurred for a short period only. The number of dead leaves was higher in N2 than in N1 ($P = 0.01$ in radish, 0.02 in rye). In N2 many older fodder radish leaves died during the frost period at the end of November. The number of living leaves on the main stem is represented by the vertical distance between the appearance and death curves, or, before the first leaves died, the appearance curve and the time axis. In rye, the number of living leaves on the main stem increased until the first leaves died, then decreased gradually. The same pattern was found for leaves on other tillers (data not shown). In
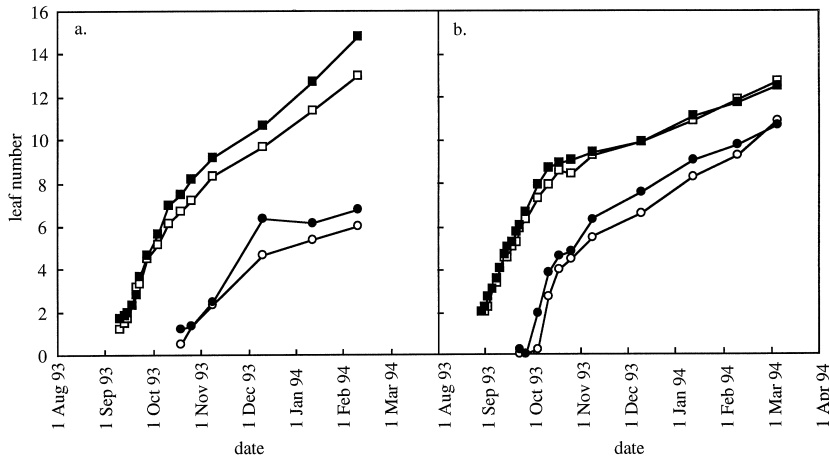
Figure 4. The number of appeared and dead leaves on the main stem in fodder radish and winter rye at N1 and N2 from August 1993 until March 1994. a. Fodder radish; b. Winter rye. Symbols: ▢ (N1), ■ (N2) = appeared leaves; ○ (N1), ● (N2) = dead leaves.

fodder radish, the number of living leaves increased until the death of the first leaf, and was almost constant afterwards, except for a temporary decrease after the frost period in November.

The leaf appearance rate on the main stem increased with temperature in both species ($P < 0.001$, Figure 5). In rye it was significantly nonlinear ($P < 0.001$). Between 2 and 5 °C, rye leaf appearance rate was constant, and it increased only above this temperature. This may have been caused by the way of calculating average temperature: by taking daily average temperatures, and then averaging over the period between measurements counting negative temperatures as 0 °C. Just above the base temperature for leaf appearance this may lead to overestimation of the leaf
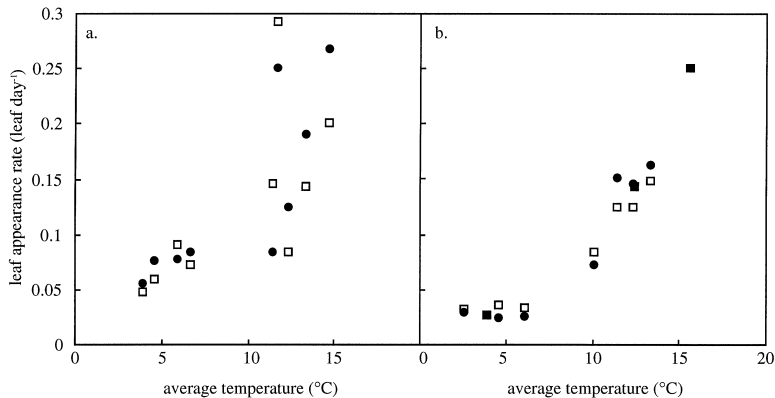

Figure 5. The leaf appearance rate as a function of average temperature during intervals of the growing season. a. Fodder radish; b. Winter rye. Symbols: ▢ = N1; ● = N2.

appearance rate, because a low daily average temperature may be the average of temperatures fluctuating around the base temperature (e.g., 3 °C may be the daily average of 8 and –2 °C or of 5 and 1 °C, which can have different effects on leaf appearance rate when the base temperature for leaf appearance rate is 0 °C). It may also be, however, that leaf appearance rate does indeed increase nonlinearly with temperature near the base temperature, or that air temperature is not exactly the same as the temperature at the site of leaf initiation.

The lifespan of the leaves expressed in degree days was 478 ± 68 °C day for fodder radish. In rye it decreased from leaf 1 (living from August until October) to leaf 9 (November-February) from 592 ± 66 to 389 ± 25 °C day at N1 and from 545 (1 observation) to 401 ± 64 °C day at N2. Deaths of the first 5 and 9 leaves were recorded in all plants in fodder radish and rye, respectively. It is not clear whether the decrease that was found in rye also occurred in fodder radish, because less of its leaves were monitored. For rye, the lifespan of N2 leaves was, on average slightly shorter than that of N1 ($P = 0.01$). The N2 rye plants had rather soft leaves that were pushed down by wind. As a result the lower leaves were strongly shaded or completely covered by the young parts of the canopy.

The natural logaritm of the number of tillers in rye was plotted against the number of appeared leaves on the main stem (Figure 6). The number of tillers increased until the appearance of the eighth leaf (mid-October), at both N supply levels. Then it remained constant until January, and then gradually decreased until the end of the experiment. The slope of the curve expresses the mean site filling, i.e. the relative increase in tiller number per leaf appearance interval. Until the appearance of the sixth leaf it was 0.629 for both N supplies, rather close to the theoretical maximum of 0.693 (Neuteboom & Lantinga, 1989). After that it decreased to zero, more rapidly in N1 than in N2, resulting in a maximum of 14 and 19 tillers per plant in N1 and N2, respectively (a significant difference, $P < 0.001$).

CO$_2$ assimilation and leaf analysis

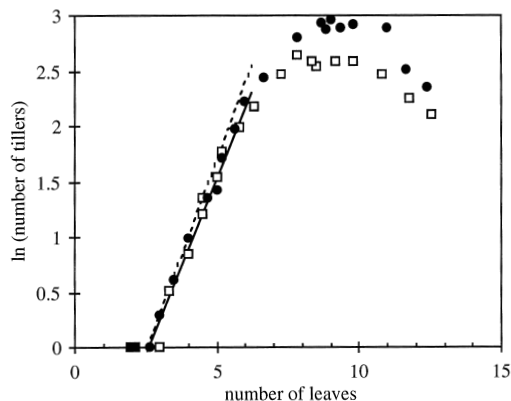A$_{max}$ was about 1.2 mg CO$_2$ m^{-2} leaf s^{-1} in September and significantly lower, about

Figure 6. The natural logaritm of the number of tillers per plant in winter rye at N1 and N2, as a function of appeared leaf number. Symbols as in Figure 5. Solid line: mean site filling; dashed line: potential site filling.
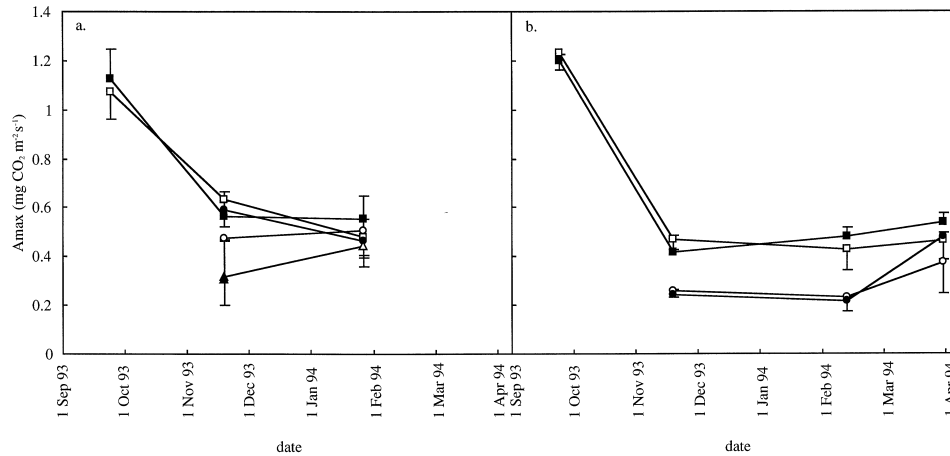
Figure 7. The light saturated CO_2 assimilation rate (A_{max}) in fodder radish and winter rye at N1 and N2 from September 1993 until March 1994. a. Fodder radish; b. Winter rye. Standard deviations are given by bars in one direction, when larger than the symbol. Symbols: —□— (N1), —■— (N2) = age 1; —○— (N1), —●— (N2) = age 2; —△— (N1), —▲— (N2) = age 3.

0.5 mg CO_2 m^{-2} leaf s^{-1} during the winter months for both species (Figure 7; $P < 0.001$). A_{max} decreased with increasing leaf age ($P < 0.001$), as previously found for potato (*Solanum tuberosum* L.) (Vos & Oyarzún, 1987) and wheat (*Triticum aestivum* L.) (Keys *et al.*, 1983). This decrease was generally small, compared to the effect of month of measurement. A_{max} was independent of N supply. There was no relation between A_{max} and leaf chamber temperature, in the range from 12 to 19 °C in September and from 5 to 15 °C in the other months (data not shown).

Leaf organic nitrogen concentrations increased slightly with N supply ($P < 0.001$) (Figure 8), especially in the older leaves. They decreased with leaf age ($P < 0.001$), from 1.5 ± 0.14 g m^{-2} at age 1 to 1.1 ± 0.16 g m^{-2} at age 2 in rye and from 1.9 ± 0.30 g m^{-2} at age 1 to 1.5 ± 0.30 g m^{-2} at age 3 in fodder radish, averaged over time and N supply levels. Nitrate concentration increased with N supply ($P < 0.001$, Figure 8) and decreased during the experiment from a high level in September to low values for both species at the end of January and to zero for rye on 4 March ($P < 0.001$). The highest value (0.47 ± 0.05 g m^{-2}) was found in radish at N2 in September.

Water-soluble carbohydrate concentrations in the leaves (Figure 9) were higher in rye than in fodder radish ($P < 0.001$) and higher in N1 than in N2. They fluctuated during the season ($P < 0.001$), with a peak in November for both species (up to 25.8 ± 1.2% and 14.8 ± 1.1% in rye and radish, respectively, at N1; percentage on a mass base) and lower values in September, January (fodder radish) and February (rye). They increased again in rye in March.
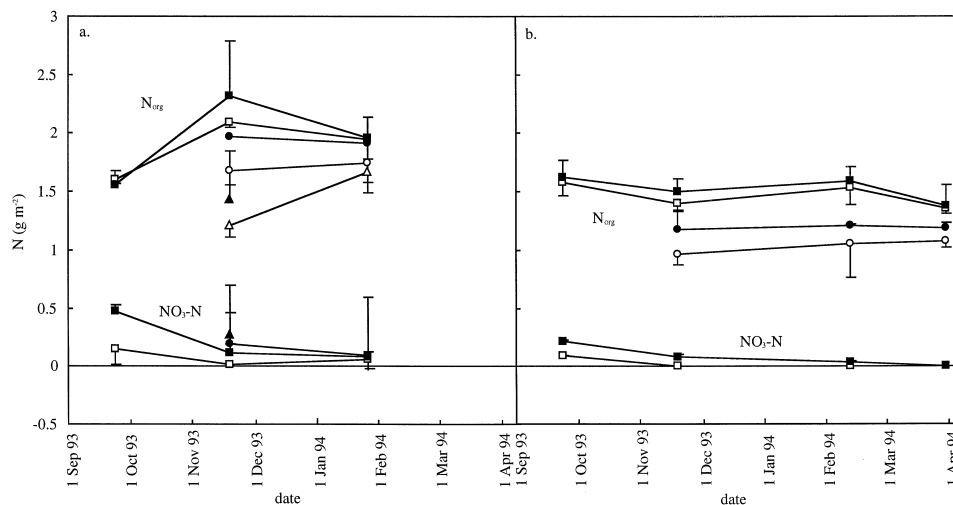
Figure 8. The leaf contents of organic (N$_{org}$) and nitrate (NO$_3^-$-N) nitrogen in fodder radish and winter rye at N1 and N2 from September 1993 until March 1994. a. Fodder radish; b. Winter rye. Standard deviations are given by bars in one direction, when larger than the symbol. Nitrate levels were zero in leaf ages 2 and 3 at N1 in Fodder radish, and leaf age 2 in rye at both N-levels. These are not included in the figure. Symbols as in Figure 7.
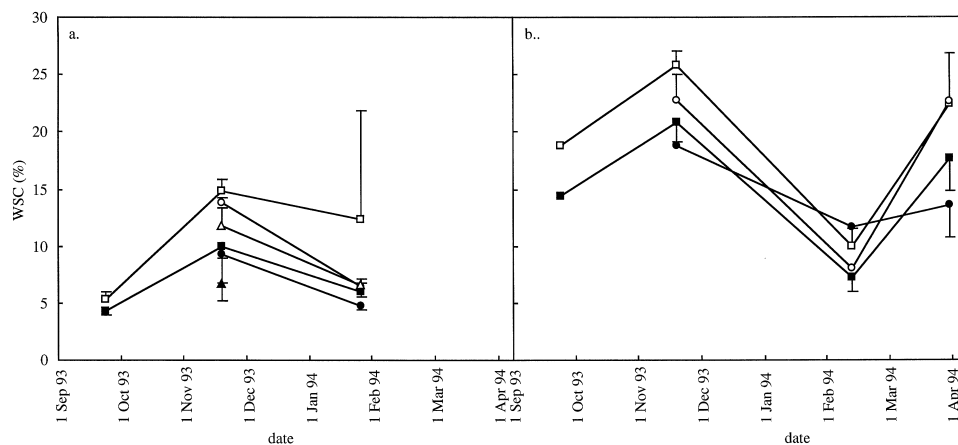

Figure 9. The leaf contents of water soluble carbohydrates (WSC) in fodder radish and winter rye at N1 and N2 from September 1993 until March 1994. a. Fodder radish; b. Winter rye. Standard deviations are given by bars in one direction, when larger than the symbol. Symbols as in Figure 7.

Model development

Relating light-saturated CO$_2$ assimilation rates to weather variables

The decrease in A$_{max}$ from September onwards, similar in rye and radish (Figure 7), might be related to the decrease in temperature and irradiance level during autumn. Both factors affect the maximum CO$_2$ assimilation rate (Huner, 1985; Jones, 1992), and may be used to estimate A$_{max}$ in varying conditions (e.g., Sheehy *et al*, 1980). Below, models are formulated to relate A$_{max}$ to temperature and irradiance during leaf development. With the models A$_{max}$ could be estimated for situations other than those of the experiment. The estimated A$_{max}$ may then be used to calculate crop growth rates in process-based simulation models.

For estimating A$_{max}$ from the growth conditions of the leaves, the period of leaf growth must be determined. This period varies during the season; it is longest in winter (Harper, 1989). Van Loo (1993) estimated the duration of leaf expansion at 1.3 times the phyllochron in perennial ryegrass. The phyllochron in rye varies from 56 to 157 °C days (Van Dobben, 1962; Hay & Abbas-Al Ani, 1983) and for radish a phyllochron of 69 and 79 °C days can be calculated for N2 and N1, respectively, from the above data. These phyllochrons take 3.6 to 10 days at 16 °C and 14 to 39 days at 4 °C, which are the extremes in average temperature per decade in this experiment (Figure 2) when frost periods are excluded. If the duration of leaf expansion is estimated according to Van Loo (1993), it will last 4.6 to 51 days. A value of 20 days is taken as an estimate; this is closer to the period of leaf growth expected at the higher than at the lower temperatures, because more leaves appear at higher temperatures. Two models are proposed that relate A$_{max}$ in young fully expanded leaves to (1) the average temperature and (2) the average irradiance intensity during 20 days before the measurement:

Model 1: A$_{max}$ = a * T
Model 2: A$_{max}$ = b * I

in which T is the average temperature (°C) and I the average global irradiance intensity during the photoperiod (J m^{-2} s^{-1}) during 20 preceding days. a and b were fitted using the A$_{max}$ data of the young full-grown leaves grown at N2. The resulting values
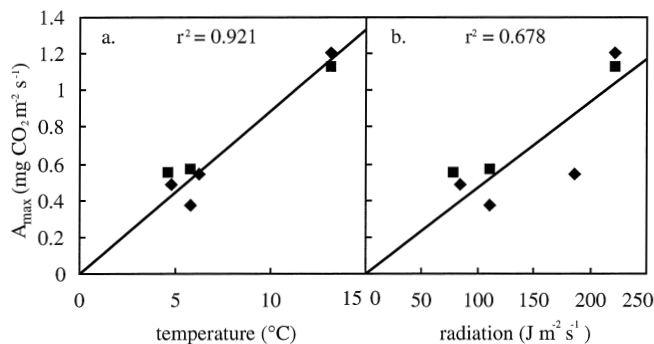

Figure 10. Fits of two models relating the light saturated CO$_2$ assimilation rate to temperature and radiation conditions during growth of the leaves. Curves are fitted models, symbols are observations. a. Model 1. b. Model 2. Symbols: ■ = radish; ◆ = rye.

were: a = 8.875 * 10^{-2} mg CO$_2$ m^{-2} s^{-1} °C^{-1} and b = 4.685 * 10^{-3} mg CO$_2$ J^{-1}. Both models had a high correlation coefficient, Model 1 even higher than Model 2. The difference in correlation is, however, determined by one point, that of the measurement on rye in March (Figure 10).

Discussion

Leaf and tiller dynamics

N2 resulted in a higher leaf appearance rate in radish (Figure 4a) and a higher number of tillers in rye (Figure 6), compared to N1. Therefore the total number of produced leaves was higher in N2 than in N1 in both species. Differences in biomass with N supply (Figure 3) are larger than differences in numbers of tillers and leaves; sizes, thicknesses and mass densities of leaf blades and sheaths and radish stems may also have been important. Sizes of the harvested leaves indicate that leaf areas were generally larger at N2 than at N1, but there was little difference in specific leaf areas (data not shown). There was an effect of N on site filling only after appearance of the sixth leaf. This is in accordance with another study in which there was no effect of N supply on site filling of young plants (*Lolium perenne* L.), unless the supply was reduced to a very low level (Neuteboom *et al.*, 1988).

The leaf lifespan in degree days was shortened at N2 in rye, which may have been due to increased shading in the denser canopy and to a higher sensitivity to frost. Vine (1983) found this higher frost sensitivity in perennial ryegrass under autumn and winter conditions.

If leaf appearance rate in rye does indeed increase nonlinearly with temperature (Figure 5b), a linear temperature sum with a certain base temperature, as often used in leaf appearance studies (e.g. Hunt & Chapleau, 1986), cannot be used unequivocally for rye. In field experiments conducted over winter, low temperatures coincide with low irradiance (Figure 2), denser canopies (more biomass, Figure 3) and shorter daylengths (data not shown), which may reduce leaf appearance rates (J.C.J. Groot, pers. comm.; Baker & Gallagher, 1983). A nonlinear relation may therefore have been a 'pure' response to temperature, or it has been confounded by interactions with other growth conditions. The differences found in the relation of leaf appearance rate and temperature, from one year or sowing date to another (Hunt & Chapleau, 1986; Hay & Abbas Al-Ani, 1983) suggest the importance of other factors.

Both leaf appearance and leaf lifespan are related to temperature. They appear to be balanced, resulting in a rather constant number of living leaves in the canopy, as was found in perennial ryegrass (Harper, 1989). The temperature sum since the appearance of a leaf appears to be an almost constant parameter for prediction of its lifespan. The decrease in temperature sum during leaf lifespan observed in rye may have been due to unfavourable winter conditions, with low irradiance levels and frost. Therefore other factors, such as shading, low irradiance levels and below-zero temperatures, should also be taken into account.

CO_2 assimilation and leaf analysis

The temperature at measurement did not influence A_{max} above 5 °C, contrary to ob-servations on rye and oilseed rape under controlled conditions (Huner *et al.*, 1986; Paul *et al.*, 1990), and temperature responses of gross CO_2 assimilation used in growth models for spring wheat, winter wheat and spring barley (*Hordeum vulgare* L.) (Spitters *et al.*, 1989; Hansen *et al.*, 1990).

Leaf development conditions affected A_{max} much more than current temperatures during the measurements. The high correlation coefficients of the models predicting A_{max} based on temperature and irradiance during leaf growth suggest that these may be useful parameters for estimating CO_2 assimilation rates of autumn and winter grown crops (Figure 10). Model 1 explained, in statistical terms, a little more varia-tion in the data than Model 2. Low temperature and low irradiance usually occur si-multaneously, however (Figure 2). The difference in correlation between Models 1 and 2 depends mainly on one point (Figure 10), so that no conclusion on the best predictive factor can be drawn from these data only. The models have to be tested in other situations to evaluate their applicability for prediction of CO_2 assimilation rates.

The difference in aboveground biomass between N1 and N2 (Figure 3) is unlikely to have been caused by differences in A_{max} (Figure 7); A_{max} differed between N1 and N2 only in the oldest leaves. The lower A_{max} in these leaves can have affected canopy CO_2 assimilation only slightly (Baker *et al.*, 1988). Since quantum yield is not either influenced by nitrogen availability (Farquhar *et al.*, 1980; Connor *et al.*, 1993; Tei *et al.*, 1993), processes other than leaf CO_2 assimilation must have limited the overall biomass production in N1 as compared to N2.

The concentration of soluble carbohydrates (Figure 9), the primary products of CO_2 assimilation, was higher in N1 than in N2, indicating that a shortage of N in N1 limited the conversion of soluble carbohydrates into structural biomass. This view is supported by the lower nitrate-N concentrations in N1 in September (Figure 8), and values near zero in N1 by November. This indicates that the conversion of soluble carbohydrates to structural biomass rather than CO_2 assimilation, limited the overall growth rate in N1. Apart from this, in N1 a greater part of the assimilates may have been allocated to the roots than in N2 (Brouwer, 1962). As a result of a lower rate of conversion to structural biomass and a possibly higher allocation to roots, the canopy may have closed later in N1, so that total light interception and canopy CO_2 assimilation were lower than those in N2.

The peak in water-soluble carbohydrate concentrations in November for both N supply levels suggests that the plants either stored carbohydrates for other use than in structural biomass, or that the rate of conversion to structural biomass was more limited than that of production of assimilates at both N supply levels. In case of the latter, this sink-limitation may have been due to nitrogen shortage in N1, but this is unlikely for N2, since nitrate was still present in the leaves in November (Figure 8). Low temperature can therefore be considered as the decisive factor, for both a limit-ed conversion rate and for storage of water-soluble carbohydrates (e.g. for preven-tion of frost damage). By January, water-soluble carbohydrate concentrations had

fallen in all treatments, after a period of short days with low irradiance, resulting in low CO$_2$ assimilation rates relative to conversion rates. In March, the water-soluble carbohydrate concentrations in the rye leaves had increased again, possibly due to the higher irradiance level.

We conclude that all measured growth-related parameters indicate that under Dutch conditions, vigorous catch crop growth takes place only before mid-November. Net growth ceases afterwards under the conditions of the experiment. Leaf appearance slows down in October and tiller production in rye stops. Light-saturated net CO$_2$ assimilation rates are halved between September and November. Assimilates accumulate in the leaves during November.

Both leaf appearance rate and leaf lifespan are related to temperature. The resulting temperature sum during leaf lifespan may be used for estimation of turnover of leaves in winter grown crops. Light-saturated CO$_2$ assimilation rates can be estimated from leaf development conditions, which may improve estimation of biomass production of autumn and winter grown crops.

Acknowledgements

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