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Original article

Influence of flooding on growth, nitrogen availability in soil, and nitrate reduction of young oak seedlings (*Quercus robur* L.)

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Abstract – Oak (*Quercus robur* L.) seedlings were grown in pots under controlled conditions and submitted to 34 days of flooding followed by two-week drainage. Roots were significantly affected with reduced extension and dry weight accumulation. After drainage, biomass production of adventitious roots markedly increased in flooded seedlings. Flooding induced a sharp decrease in NO_3^--N content in the soil especially in the bottom of pots. NH_4^+-N concentrations increased significantly but at less level compared to NO_3^--N decreases. During flooding, root nitrate reductase activity (NRA) was similar to controls while leaf NRA was always below that of controls. The flooded roots maintained amino acid synthesis despite the nitrate depletion in soil. By contrast, leaf amino acid content decreased significantly in flooded seedlings especially at day 34. In flooded seedlings, the transfer of amino acid from cotyledons was disrupted but the transfer capacity was restored after drainage. Relationships between nitrate reduction and changes in soil mineral nitrogen availability are discussed.

flooding / mineral nitrogen availability / nitrate reduction / *Quercus robur* L.

Résumé – Influence de l'ennoyage sur la croissance, la biodisponibilité en azote du sol, et sur la réduction des nitrates chez de jeunes semis de chêne pédonculé (*Quercus robur* L.). Des semis de chêne pédonculé (*Quercus robur* L.) cultivés en conditions contrôlées ont été soumis à 34 jours d'ennoyage suivi de deux semaines de drainage. La longueur des racines stressées ainsi que leur accumulation de biomasse ont été significativement réduites. Après drainage, la biomasse des racines adventives des semis ennoyés a fortement augmenté. L'ennoyage a induit une forte diminution des teneurs en N-NO_3^- dans le sol, particulièrement dans les 5 cm inférieurs du pot. La concentration en N-NH_4^+ a augmenté significativement mais sans compenser la diminution de N-NO_3^-. Pendant l'ennoyage, les activités nitrate réductase racinaires des deux lots étaient similaires alors que dans les feuilles elle était toujours inférieure chez les plants ennoyés. Les racines ennoyées ont maintenu la synthèse d'acides aminés malgré la disparition des nitrates dans le sol. Au contraire, la teneur en acides aminés dans les feuilles avait significativement diminué dans les semis ennoyés en particulier à 34 jours. Dans les semis ennoyés, le transfert des acides aminés depuis les cotylédons était perturbé, cependant après drainage la capacité de transfert était rétablie. Les relations entre la réduction des nitrates et les changements de la biodisponibilité de l'azote minéral du sol sont discutées.

ennoyage / azote minéral disponible / réduction des nitrates / *Quercus robur* L.

1. INTRODUCTION

Flooding is characterised by a temporary or a permanent saturation of soil pores with water. This reduces drastically gas diffusion and leads to hypoxic conditions. Soil inundation occurs in irrigated soils and heavy rainfall areas and depends therefore on soil characteristics, climatic parameters and human activities [4, 33]. Thus, inundation induces a decrease in plant production both in agricultural and forest areas. During flooding, many physical, chemical and biological processes change that may alter the capacity of soil to support plant growth [22]. Soil micro-organisms use, in much case, oxygen as the terminal electron acceptor for degradation of organic compounds. Under flooding, nitrate replaces oxygen as the terminal electron acceptor in microbial respiration leading to denitrification and/or nitrate ammonification [23]. In fact, three nitrate reducing pathways are known in soils: (1) dissimilatory nitrate reduction to ammonia (DNRA or NH_4^+ accumulators), (2) nitrogen dissimilating bacteria which are only able to reduce nitrate to nitrite (NO_2^- accumulators) and (3) denitrifyiers that are able to reduce nitrate to nitrous oxide or to dinitrogen [5, 7, 27]. Bacterial reduction of nitrate is considered to be an important loss of available nitrogen from soils [3, 19]. This process induces a competition for nitrate between root and bacteria [28]. Thus, flooding influences nutrient uptake by plants [11, 24]. Under anaerobic conditions, morphogenesis and several metabolic pathways, like nutrient uptake, photosynthesis, and respiration are slowed down or altered [21]. In woody species, earlier studies have shown that *Quercus robur* seedlings are particularly tolerant to hypoxia [9, 13, 14, 32, 36] partly because of their

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greater capacity of producing new roots in the vicinity of normoxic soil layers [13, 32]. Roots are the plant organs that suffer most frequently from low oxygen stress and this could disturb uptake processes and particularly nitrate uptake. Nitrate reductase is the main enzyme implied in nitrate reduction steps. Its synthesis is promoted by environmental factors, especially light and nitrate availability, via phytochrome and photosynthetic sugar production. In some species, root nitrate reductase activity increases under hypoxic conditions [12]. Nitrate reduction can act as a sink for protons, thus limiting damaging cytoplasmic acidosis [15]. Some earlier studies showed that nitrate supply was able to improve the growth of wheat and barley and alleviate to some extent the effects of anaerobic conditions [2]. The aim of the present study was to determine whether soil nitrate depletion could affect the absorption and reduction of nitrate in roots and leaves and the amino acid partitioning in *Quercus robur* seedlings under soil hypoxia.

2. MATERIALS AND METHODS

Oak seedlings (*Quercus robur* L.) were grown from acorns collected during autumn 2002, under an individual oak tree (Courcelle Forest, Montbéliard, France). Acorns were stratified at 1 °C. Clear acorns were sown in pots filled with moist vermiculite. Two weeks later, when the primary roots were 5–7 cm long, the seedlings were transplanted into 2.5 L, 25 cm deep pots (one seedling per pot) filled with a 6 /3 /1 (v/v/v) soil forest, sand and black peat mixture. Forest soil was harvested in Chaux (Jura, France) forest site and was ground and sieved through a 4 mm mesh screen. Seedlings were grown in controlled conditions at 22 °C during the day (6.00–20.00 h) and 14 °C at night with 60% relative humidity. Photon flux density was provided by halogen lamps (HQI-T, 400 W) and sodium lamps (250 W) resulting in a photosynthetic photon flux density of 300 μmol m^{-2} s^{-1} at the height of the seedlings.

2.1. Experimental design and sampling procedure

Flooding (F) was imposed by filling the outer container (40, 36, 23 cm) with soil mixture and deionised water up to 2–3 cm below soil surface. In the control treatment (C), seedlings were watered at 75% of field capacity. For both treatments, pots were prepared and maintained for one month until the transfer of the germinated seedlings. This allowed reduced conditions to be established in the soil before seedling establishment (day 0). All pots were randomly placed on a bench in the growth chamber and rearranged every 3–4 days. Following transfer of seedlings to control and flooded pots (day 0), plants were harvested at days 15, 26 and 34 respectively, during flooding period ($n = 5$ of each date).

Forty days after seedling transfer, pots were drained and seedlings left growing for 14 days. They were then harvested at day 54. During the whole experiment, Eh (redox potential in mV) was monitored with a combined platinum electrode connected to WTW instrument (Germany).

2.2. Soil sampling

Mineral composition of the soil mixture was analysed before the start of the experiment (pH: 6.5, mineral elements expressed as mg gDW^{-1} of soil mixture: 0.10 NO_3^-, 0.01 NH_4^+, 9.60 Ca^{2+}, 1.34 K^+, 0.15 Na^+, 12.44 Fe and 0.40 Mn). Then at each harvest period, soil samples were collected at 5-cm of the top, at 5-cm of the bottom and around roots (rhizospheric soil) in each pot. All samples were stored in polyethylene bags and then kept at 4 °C until bacteria analysis or at –20 °C until chemical analysis.

2.3. Numeration of denitrifying and of total cultivable microflora

Denitrifying bacteria were numerated with a most-probable-number (MPN) procedure [37] using NB Medium and Griess-Ilosvay reagent as described by Garcia et al. [17]. For each sample, MPN was calculated with MPN Calculator 4.04 software using standard Mac Crady tables. Results were expressed as numbers of bacteria g^{-1} soil dry weight. Total cultivated microflora was numerated in Petri dishes with Nutrient Broth (N.B., DIFCO) medium. After 48 h of aerobic incubation at 28 °C, the growth of microbial populations was estimated with the colony-forming units (CFU) method. Frequency of denitrifying bacteria was expressed as the ratio of denitrifying bacteria to total cultivated microflora (%).

2.4. Growth parameters and shoot water potential

During each harvest, shoot water potential was measured with a Scholander pressure chamber, and stem length and leaf area of each growth flush were monitored. Leaf area was measured using a Li-3000A portable leaf area meter (Licor Inc.). Root length was also measured before nitrate reductase assays. All samples were weighed to estimate fresh weight and were separately lyophilised before being ground to fine powder.

2.5. Nitrate and ammonium determinations

Soil mineral nitrogen was extracted by incubating 10 g of fresh soil with 20 mL of demineralised water during 60 min at 50 °C. The solution was centrifuged at 2500 rpm during 15 min and the resulting supernatant was filtered. The fine powder of roots and leaves was also submitted to the same process using 50–200 mg of powder and 5 mL of demineralised water [34]. Nitrate and ammonium concentrations were measured photometrically by automatic SKALAR (column 27693 for NO_3^- at 540 nm and column 27067 for NH_4^+ at 660 nm).

Nitrate reductase activity (NRA) was assayed in vivo both in leaves and young roots at each harvest period according to the modified method from Thomas and Hilker [34]. For each seedling, 100 mg of fresh leaf discs or 50 mg of root tips (pieces of ca. 2 mm length after rinsing with deionised water) were sampled. Prior to incubation, the samples were kept on ice and protected from light to prevent a premature onset of NO_3^- reduction. Samples were then infiltrated for 10 min under vacuum with 5 mL assay medium containing 0.4 M KH_2PO_4 (pH 7.5) and 1.5% 1-propanol. Assay was started by adding 0.5 mL of 0.05 M KNO_3 and the samples were incubated at 30 °C during 90 min in darkness, the reaction was stopped by boiling during 5 min. 1 mL assay was added to a mixture of 1% sulfanilamide in 3 M HCl, 1 mL aqueous 0.1% N-naphthlethylene diamine dihydrochloride and 1 mL of deionised water. After 10 min of incubation in the dark, absorbance was measured at 540 nm. Controls were obtained by incubation without KNO_3. Nitrate reductase activity was expressed in nmol NO_2^- g_{FW}^{-1} h^{-1} and in nmol NO_2^- plant organ g^{-1} h^{-1} (root or leaves). Free amino acids were assayed after ethanol extraction using ninhydrine reagent according to Moore and Stein method [25].

2.6. Statistics

The results are given as means with standard errors. Comparisons ($n = 5$) between different treatments were performed with the Mann-Whitney test by using the StatView software (SAS Institute, Clary, NC, USA). The significance level was 5% (* $p < 0.05$) and 1% (** $p < 0.01$).

Table I. Organ biomass (g dry weight), total leaf area and shoot water potential of control and flooded *Quercus robur* seedlings harvested during flooding exposure and 14 days after drainage (day 54). Mean ± SE, $n = 5$, (*) and (**) indicates significant differences between flooded and control at $p < 0.05$ and $p < 0.01$ respectively; (Mann and Whitney test). For each treatment, different letters indicates a significant cotyledons biomass decrease between day 0 and the others harvest days 15, 26, 34 or 54, (b') and (b'') indicate significant differences at $p < 0.05$ and $p < 0.01$ respectively; (Mann and Whitney test).

		Day 0	Day 15	Day 26	Day 34	Day 54
Cotyledons (g)		2.62 ± 0.46^a				
	Control		2.19 ± 0.63^a	1.71 ± 057$^{b''}$	1.13 ± 0.27$^{b''}$	0.77 ± 0.23$^{b''}$
	Flooded		2.11 ± 0.43^a	2.02 ± 0.99^a	1.66 ± 0.68$^{b'}$	1.35 ± 0.55$^{b''}$
Taproot (g)		0.05 ± 0.01				
	Control		0.33 ± 0.09	0.62 ± 0.1	1.00 ± 0.19	2.02 ± 0.54
	Flooded		**0.12 ± 0.04****	**0.26 ± 0.09***	**0.34 ± 0.06****	**0.74 ± 0.14****
Lateral roots (g)		0				
	Control		< 0.001	0.23 ± 0.05	0.26 ± 0.14	0.54 ± 0.22
	Flooded		< 0.001	**0.08 ± 0.02****	**0.08 ± 0.01****	0.53 ± 0.19
Stem first flush (g)		0				
	Control		0.12 ± 0.04	0.28 ± 0.06	0.43 ± 0.12	0.75 ± 0.27
	Flooded		0.09 ± 0.05	0.21 ± 0.09	0.35 ± 0.08	0.54 ± 0.12
Stem second flush (g)		0				
	Control		0	0.003 ± 0.0	0.02 ± 0.01	0.22 ± 0.08
	Flooded		0	0	0.003 ± 0.0	0.05 ± 0.04
First flush leaves (g)		0				
	Control		0.11 ± 0.03	0.28 ± 0.09	0.33 ± 0.13	0.30 ± 0.13
	Flooded		0.10 ± 0.02	0.18 ± 0.07	0.20 ± 0.03	0.26 ± 0.04
Second flush leaves (g)		0				
	Control		0	0	< 0.001	0.34 ± 0.14
	Flooded		0	0	0	**0.09 ± 0.1****
Total leaf area (cm^2)		0				
	Control		43.5 ± 9.7	110.2 ± 27.1	120.9 ± 21.5	225.2 ± 35.7
	Flooded		34.1 ± 11.1	95.1 ± 17.8	**76.8 ± 13.5****	123.4 ± 35.0
Shoot water potential (MPa)						
	Control		–0.46 ± 0.18	–0.63 ± 0.11	–0.27 ± 0.12	–0.34 ± 0.13
	Flooded		**–0.94 ± 0.11***	–0.58 ± 0.07	–0.26 ± 0.11	–0.27 ± 0.13

3. RESULTS

At the beginning of the flooding treatment (30 days before seedling transfer), redox potential (Eh) values averaged +280 mV (Fig. 1). Thereafter, Eh decreased to about 200 mV at day 0 when seedlings were transferred to pots. Eh values continued to decline and reached –90 mV just before drainage.

3.1. Growth parameters and shoot water potential

Taproot and lateral root biomass (g DW) were significantly decreased by flooding while stem biomass of first and second flushes remained unaffected (Tab. I).

Root biomass accumulation was strongly decreased during flooding and reached only about 34% of the control at day 34. At day 54, 14 days after drainage, lateral roots of the flooded seedling showed a large increase in biomass and reached 98% of the controls. The root growth resumption after drainage was very important. However taproot biomass remained significantly below the control seedlings (36% of the control). First flush leaf biomass was unaffected by flooding, while those of second flush were severely affected (Tab. I). Total leaf area was significantly reduced at day 34 only, and regained values similar to controls after drainage. Throughout the experiment, no leaf necrosis was detected. Cotyledons of control seedlings

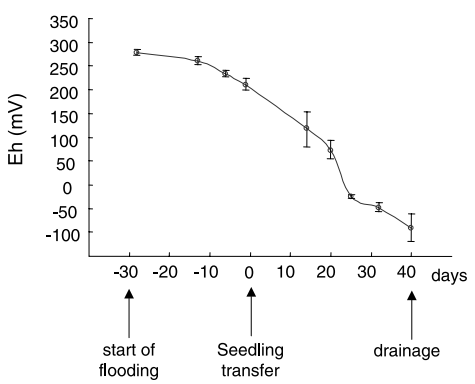

Figure 1. Time course of redox potential during flooding exposure. Data are the means (± SE) of 5 replicates.

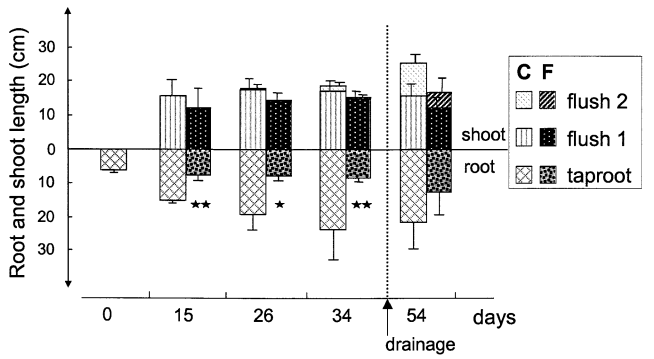

Figure 2. Mean root and stem length (cm) of flooded (**F**) and control (**C**) *Quercus robur* seedlings harvested during flooding and 14 days after drainage (day 54). Mean ± SE, $n = 5$, (*) and (**) indicate significant differences between flooded and control at $p < 0.05$ and $p < 0.01$ respectively; (Mann and Whitney test).

showed a gradual and highly significant decrease in biomass after day 26 (difference between day 0 and the others harvest days). In flooded seedlings, it decreased significantly after day 34 only (Tab. I). With the exception of the harvest at day 15, no obvious effect of flooding was detected on shoot water potential. During flooding, root length was significantly reduced while shoot length was unaffected. After drainage, root length increased to reach a value close to that observed in controlled seedlings (Fig. 2).

3.2. NO_3^--N and NH_4^+-N content changes

The NO_3^--N pool was measured in top (5 cm) and bottom (5 cm) soil layer of the pots (Fig. 3). NO_3^--N concentration was always lower in bottom compared to top soil. In controls at the top nitrate content rose until day 26 and decreased thereafter. Flooding had a marked effect on soil nitrogen especially for NO_3^--N. At day 34, the level of NO_3^--N concentration in flooded soil, was 7-fold and 50-fold lower, in the top and in the bottom of pots respectively, than in controls. After drainage, NO_3^--N content displayed a slight increase. Unlike NO_3^--N, NO_4^+-N changes were less marked between top and bottom soil (Fig. 3). Thus, during flooding, NO_4^+-N concentrations, especially in top soil layers, increased significantly but did not compensate the NO_3^--N decreases. After drainage, no significant difference was observed.

Flooding also induced a sharp decrease in nitrate content in rhizospheric soil and in taproots. This decrease was more pronounced in rhizospheric soil (Fig. 4). After drainage, the amount of nitrate measured in the flooded rhizospheric soil tended to increase while that of taproot decreased. In parallel, ammonium content in flooded rhizospheric soil increased significantly at day 26 and day 34. After drainage, ammonium concentration became similar between the two treatments.

Whatever the treatment, ammonium concentrations in taproot and nitrate concentrations in leaves remained below the detection threshold of the colorimetric method.

3.3. Numeration of bacteria

The number of total culturable bacteria was unaffected by flooding. Values ranged from 2.4 to 5.5 10^9 and from 1.5 to 4.5 10^9 CFU g^{-1} dry soil in control and flooded treatments, respectively. In bulk soil, percentage of denitrifying bacteria was similar in control and in flooded treatments (19% and 12%, respectively). However, this percentage was higher in flooded rhizospheric soil (30%) than in controls (5%).

3.4. Nitrate reductase activity (NRA)

During flooding, NRA (nmol NO_2^- g_{FW}^{-1} h^{-1}) was not significantly different in roots of flooded and control seedlings

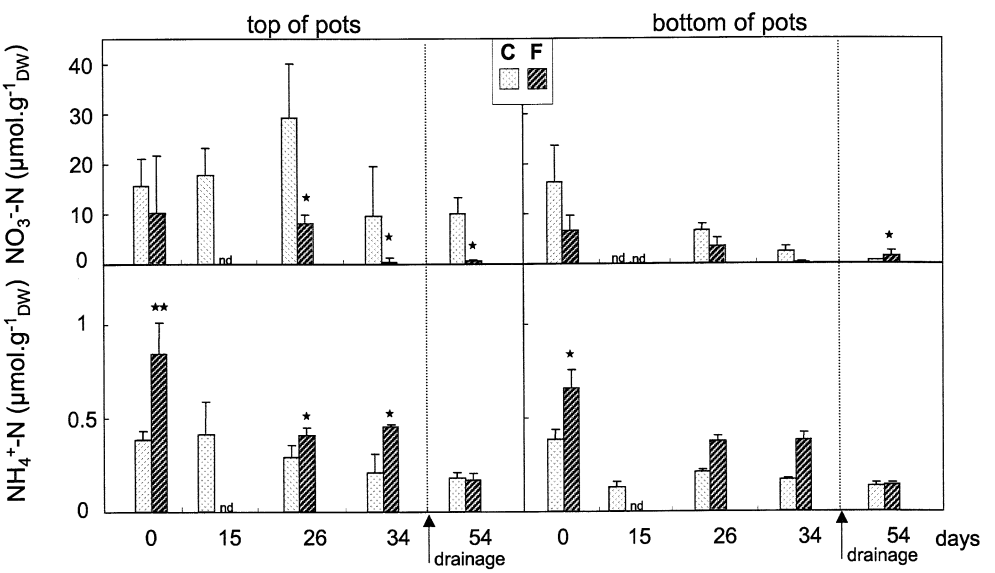

Figure 3. NO_3^--N and NO_4^+-N contents measured in the top 5cm and in the bottom 5cm soil layer of pots. Measurement were performed after each harvest in 5 pots per treatment (**C** and **F**) during flooding and 14 days after drainage (day 54). Mean ± SE, $n = 5$, (*) and (**) indicate significant differences between flooded and control at $p < 0.05$ and $p < 0.01$ respectively; (Mann and Whitney test). Nd, not determined.

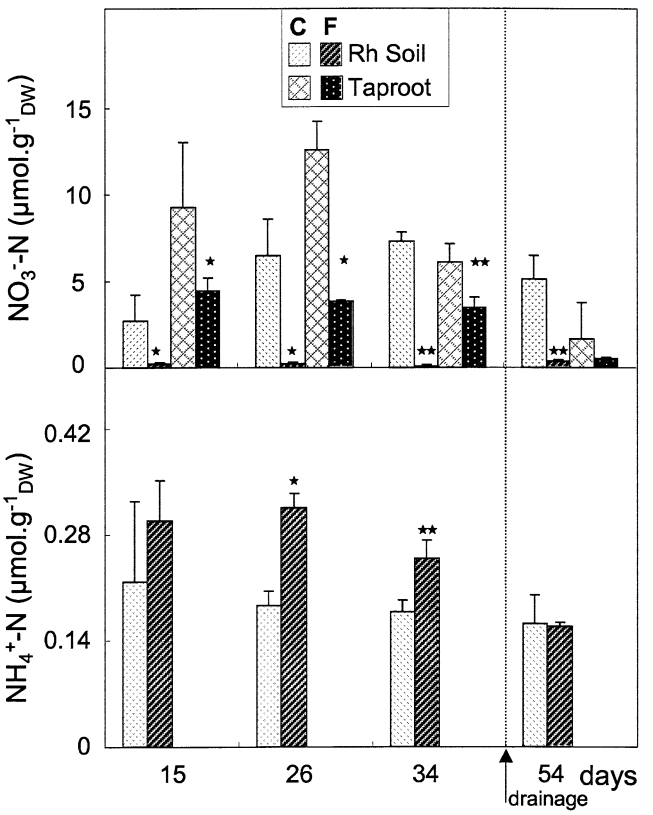

Figure 4. NO_3^--N and NO_4^+-N contents measured in the rhizospheric soil (Rh) and in the corresponding taproot of flooded (**F**) and control (**C**) *Quercus robur* seedlings harvested during flooding and 14 days after drainage (day 54). Mean ± SE, $n = 5$, (*) and (**) indicate significant differences between flooded and control at $p < 0.05$ and $p < 0.01$ respectively; (Mann and Whitney test).

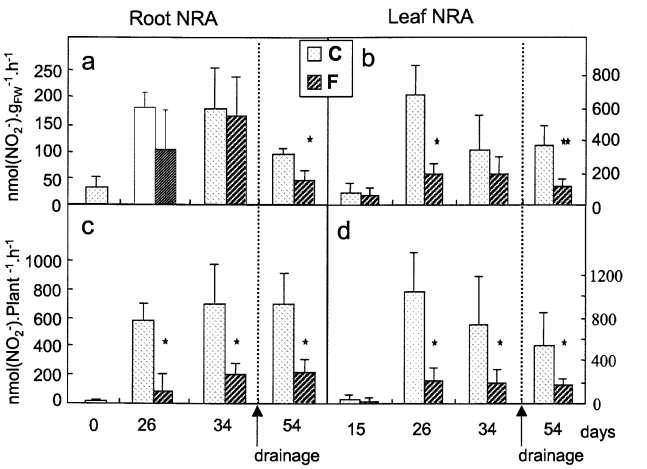

Figure 5. Nitrate Reductase Activity (nmol NO_2^- g_{FW}^{-1} h^{-1}) measured in fine roots (**a**) and in first flush leaves (**b**) of flooded (**F**) and control (**C**) *Quercus robur* seedlings harvested during flooding and 14 days after drainage (day 54). Results are also presented per plant organ (**c**, **d**) (nmol NO_2^- Plant organ^{-1} h^{-1}). Mean ± SE, $n = 5$, $n = 3$ in flooded root at days 0, 26 and 34, $n = 4$ in control roots at days 26 and 54. (*) and (**) indicate significant differences between flooded and control at $p < 0.05$ and $p < 0.01$ respectively; (Mann and Whitney test).

(Fig. 5a). However, after drainage, NRA decreased significantly in flooded roots. NRA (nmol NO_2^- g_{FW}^{-1} h^{-1}) showed a significant 3-fold decrease in whole root system of flooded seedlings in comparison to controls (Fig. 5c). In the first flush leaves of controls, NRA reached a maximum at day 26 and decreased to a stable level until day 54 (Fig. 5b). In stressed seedlings, leaf NRA showed a similar time course to controls but with a weaker amplitude. In fact, leaf NRA was always lower in flooded than in control seedlings with significant effect at day 26 and day 54. Expression of leaf NRA per total biomass of leaves showed a strong effect of flooding on total leaf capacity to reduce nitrate (Fig. 5d). Indeed, leaf NRA assessed in flooding seedlings was 5-fold to 3-fold lower than that of control seedlings (Fig. 5d).

3.5. Total amino acids

The total amino acid content were similar in flooded an in control tap roots (Fig. 6). However, after drainage this amino acid pool decreased below that of control taproots (Fig. 6). On account of a small quantity of flooded lateral roots, their amino acid content was measured only after drainage (day 54). It was similar to that of controls (Fig. 6). In the first flush leaves,

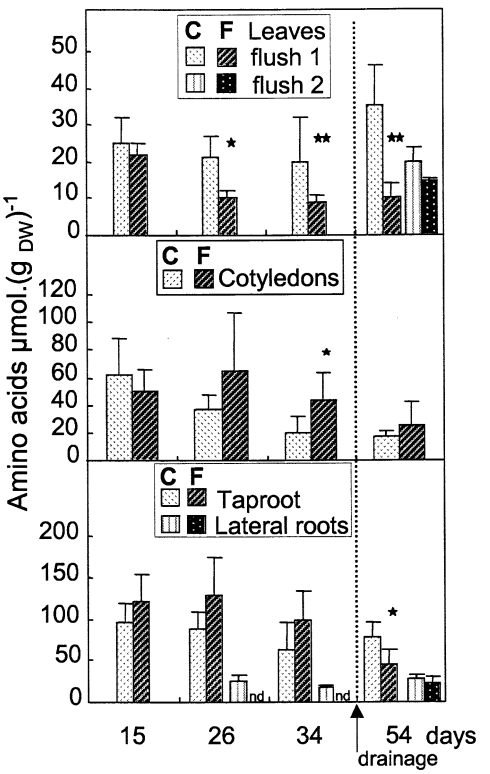

Figure 6. Amino acid contents in leaves, cotyledons and roots of flooded (**F**) and control (**C**) *Quercus robur* seedlings harvested during flooding and 14 days after drainage (day 54). Mean ± SE, $n = 5$, (*) and (**) indicate significant differences between flooded and control at $p < 0.05$ and $p < 0.01$ respectively; (Mann and Whitney test).

flooding induced a 2-fold decrease of total amino acid concentration at day 26 and day 34 respectively. After drainage, in the first flush leaves of stressed seedlings, amino acid content remained significantly lower in comparison to control seedlings (Fig. 6). However amino acid content of the second flush leaves was similar between the two treatments (Fig. 6). The cotyledon amino acid containt of flooded seedlings remained slightly higher than in control but at day 34 of stress exposure the flooded cotyledons contained 2-fold higher amino acid concentrations than that of control cotyledons (Fig. 6). After drainage the flooded cotyledon amino acid content was similar to that of the control seedlings (Fig. 6).

4. DISCUSSION

4.1. Growth and shoot water potential

Flooding severely affected the root system (decreased length and biomass), but not stems and leaves of young seedlings of *Quercus robur*. This is consistent with earlier results showing that the root is the first target of growth inhibition during flooding [22, 35]. This reduced root growth is largely attributed to a decreased of O_2 concentration in the rhizosphere [22]. In the shoot, the observed decrease in total leaf area and in stem length at the third harvest (day 34) was due to the delay in bud break of the second flush. This could be due to a decrease in cytokinin synthesis resulting from a reduction in root tip biomass in flooded seedlings. In fact, Dickson [10] underlined the involvement of cytokinin in bud break initiation of *Quercus rubra* seedlings. Initiation of new roots in stressed seedlings began 26 days after flooding exposure, length and biomass production of these adventitious roots increased after drainage. Similar results have been reported for *Quercus robur* submitted to waterlogging [9, 14, 32]. Water potential remained similar between treatments except at day 15. This would be a consequence of the first necrosis and few branching observed in root system of the flooded seedlings. The recovery of shoot water potential close to controls could be due to initiation of new root formation at day 26. Earlier studies have shown a depressing effect of flooding on water potential and on hydraulic conductance in root [13, 32]. In contrast, Ahmed et al. [1] showed that flooding did not affect water potential of mungbean plants.

4.2. NO_3^--N and NH_4^+-N content changes

Flooding induced a sharp decrease in soil NO_3^--N, especially in the bottom of pots. N turnover in soil is characterised by a coupling between nitrification and denitrification. Many authors [5, 16] have shown that nitrification is restricted to the 5 cm oxic surface layer while denitrification occurs in the lower hypoxic layers. Similar differences between top and bottom of pots were observed in our experiment. Nitrate decrease could be explained by an increase in denitrification when the redox potential dropped. In fact, aerobic nitrifying and anaerobic denitrifying bacteria are very sensitive to soil water content. Aerobic processes occur when 20 to 60% of the pores are filled with water. Above 60%, anaerobic processes such as denitrification increase while nitrification decreases rapidly [20]. Ponnamperuma [29] reported that under hypoxia, denitrification was the main cause of the depletion of nitrate. No analyses of nitrous oxide were performed in this work, but the slight increase in NO_4^+ contents (and in NO_2^- contents data not shown) in the soil during the present experiment cannot have counterbalanced the sharp decrease in NO_3^--N. The obtained results allow us to suppose either that denitrifying bacteria could represent a major group of nitrate reducing bacteria or that Dissimilatory Nitrate Reduction to Ammonia (DNRA) could be the main denitrification process. In this second case, a large part of produced ammonium could be taken up by root for amino acid synthesis.

In the flooding treatment, the decrease of nitrate concentrations was more important in the rhizosphere than in the bulk soil (Figs. 3 and 4). These results were correlated with an increase in the percentage of denitrifying bacteria. This could influence N-turnover in soil and thereafter nitrogen assimilation pathways in roots.

4.3. Nitrate reduction

Nitrate reduction can occur both in roots and shoots but the relative contribution of the two compartments may vary depending on species and on nitrate levels in soil. Nitrate reduction assessed in control seedlings by nitrate reductase activity was higher in leaves than in roots. These results are consistent with those of Thomas and Hilker [34]. During flooding, root nitrate reductase activity was similar to controls. These results highlight the ability of flooded seedlings to maintain nitrate reductase activity in roots despite low nitrate content in soil. In some species, nitrate reductase activity of the root was even increased under hypoxic conditions [12, 26]. This increase in nitrate reduction can act as a proton sink, thus helping to avoid damaging cytoplasmic acidosis [15]. However, if we considered.

NRA at the scale of the whole root biomass, significantly lower NR activities appear in roots of stressed seedlings at all harvesting times. In that case, these lower activities could be ascribed to a reduction in root biomass. Indeed, nitrate content in the roots of stressed seedlings showed a significant decrease in comparison to control seedlings. This decrease could account for both a decrease in total root uptake area and a sharp drop in soil nitrate content after an enhancement of denitrification processes.

Nitrates which are not reduced or stored in roots can be translocated via the xylem to be reduced in leaves. Foliar nitrate reductase activity is age dependent: the maximum activity occurs when the rate of leaf expansion is maximal. Thereafter, the activity declines rapidly [31]. In our experiment, foliar nitrate reductase activity measured in the control seedlings showed similar changes depending on leaf developmental stages. However, nitrate reductase activity in leaves of flooded seedlings was always below that of controls. This weak nitrate reductase activity in the leaves of stressed seedlings could be due to a decrease in nitrate import from root. Nitrate induces activation and induction of nitrate reductase. Thus, the observed decrease of nitrate reductase activity in leaves of flooded seedlings could be related to a low nitrate translocation from the root. Total nitrate reductase activity in control seedlings was even larger especially during the last harvest (Fig. 5d). Unlike our results,

Quercus seedlings grown on sand showed maintained nitrate reductase activity in roots and leaves in flooded and control plants [34]. However, these authors supplied nutrient solution (4 mM of NH_4NO_3 or KNO_3) to potted seedlings 24 h before the nitrate activity assays. This supply may have increased nitrate reductase activity in stressed seedlings and occulted the real effect of flooding via nitrogen availability in the soil.

During flooding, amino acid contents in taproot of stressed seedlings remained high and similar to that of controls. This result indicated that flooded roots maintained amino acid synthesis despite the decrease in soil nitrate. By contrast, ammonium increased during flooding due to denitrification (Fig. 4). This ammonium was probably absorbed and used for amino acid synthesis in roots via the glutamine synthetase pathway. Reggiani et al. [30] have shown that glutamine synthetase and ferredoxin-dependent glutamate synthase are synthesized during anoxia in rice roots. These findings indicate that the glutamine synthetase/glutamate synthase cycle could play an important role in amino acid accumulation under hypoxia. Root growth of *Quercus robur* seedlings was severely reduced during flooding with as a consequence a smaller mobilisation of cotyledon reserves. Unlike in roots, after full expansion, leaf amino acid content decreased significantly in flooded seedlings especially at day 34 of flooding. This decrease could be the outcome of reduced nitrate assimilation in leaves and /or a decrease in import capacity of amino acids from source organs (taproot and cotyledon). At the end of flooding (day 34), reduction in leaf expansion may have altered source-sink relationships leading to a significant decrease in leaf amino acid content. The transfer of resources from cotyledons to growing seedlings seems to be almost complete at a very early stage [18]. In our experiment, under flooding stress, the decrease in biomass and amino acid content of cotyledons was more pronounced in control seedlings than in stressed seedlings. These results indicated that resource transfer from cotyledon to growing organs was disturbed even six weeks ago after shoot emergence. In fact, according to García-Cebrián et al. [18], the extent of transfer reaches 80% of the biomass and 73% of the nitrogen content of the cotyledon respectively only 14 days after shoot emergence.

After drainage, amino acid transfer seemed to recover in stressed seedlings because new formed organs, e.g., second flush leaves and adventitious roots, had similar amino acid contents to those of control seedlings. These changes could be due to an activation of nutrient transfer from cotyledons.

In conclusion, the present study provides evidence that nitrate reduction and amino acid partitioning was impaired by flooding especially in leaves. Further experiments will be helpful to clarify (i) the role of ammonium assimilation pathway in maintaining amino acid content in root (ii) differences between *Quercus* sp. seedlings showing various levels of tolerance to Hypoxia in relation to the possible contribution of nitrate reduction in avoiding cytoplasmic acidosis.

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