

CHAPTER 1: Literature review

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In the search to understand functioning of nitrogen-limited ecosystems, knowledge has been sought about plant traits which enhance competitive acquisition of N (e.g. Näsholm *et al.*, 1998; Robinson and Van Vuuren, 1998). At the same time, surveys (e.g. Monk, 1966) have established a correlation between the occurrence of evergreen plants and that of nutrient-poor environments, i.e. mostly nitrogen or phosphorus limited. Consequently, It has been hypothesised that species with long-lived leaves are better adapted to infertile environments than species with short-lived leaves. Research has then tried to explain which traits associated with leaf longevity might facilitate such adaptation. In this chapter the current knowledge on this topic will be reviewed focusing on nitrogen as the limiting nutrient.

I Implication of leaf longevity for plant growth

I.1 Definition of leaf longevity and leaf habit

Leaf longevity, i.e. leaf life span, can range from a few weeks in ephemerals to over 20 years in conifers (Chabot and Hicks, 1982). It can be defined as the length of time during which one cohort of leaves remains on the plant. Leaf life span is closely linked with the leaf phenology of the plant and ultimately its leaf habit, i.e. whether the plant is deciduous or evergreen. Leaf habit is determined by the proportion of the year during which the plant retains its leaves. Deciduous species retain their leaves for less than one year. The length of the growing season and climatic conditions largely determine their leaf life span (Eckstein *et al.* 1999). In temperate climates, these species are leafless in the winter season, whereas in tropical or subtropical climates these species are leafless during the dry season. Evergreen species retain their leaves for more than one year. Intermediate patterns are those of semi-deciduous species (e.g. Russell, 1936) or wintergreen species (as defined by Bell and Bliss, 1977), which bear their leaves for one full year. Species in these categories replace each cohort of leaves with the next annual cohort at the start of the next growing season (e.g. *Nothofagus fusca*). Others species are facultatively deciduous and shed their leaves in response to environmental stresses such as drought (e.g. *Artemisia californica*). Such patterns of leaf habit are however restricted to species growing in climates with no marked seasonal variations of temperature or water availability.

1.2 Adaptive significance of leaf life spans

Several hypotheses have been advanced to explain the dichotomy between deciduous and evergreen species. These have been comprehensively reviewed (e.g. Chabot and Hicks, 1982 and Chapin, 1980). Four major adaptive significances of leaf life span have been recognised as addressing key issues for the understanding of a wide range of ecosystems: drought avoidance or tolerance, herbivore deterrence, improvement of plant carbon balance and nutrient conservation.

Evergreenness as an adaptive trait to enhance nutrient conservation in infertile soil has been mainly proposed based upon the studies by Monk and Beadle. Monk (1966) studied the composition of seven different forest communities in North-central Florida. He assessed the “evergreen character of a stand” - that is, the proportion of trees in the stand belonging to evergreen species – and found that it was inversely correlated with edaphic factors such as soil nutrient content (including K and P but not N) or moisture. Monk further suggested that evergreenness involved a nutrient-conservation mechanism. Beadle (1954) suggested that the repartition of Australian xeromorphic (including deciduous species) and sclerophyllic species (only evergreen species) was driven by soil phosphate availability. Although the relevant data happen to be available (e.g. Rodwell, 1991) there is to my knowledge no quantitative analysis of the distribution of evergreen versus deciduous species in relation to soil fertility for European plant communities. However, it is commonly accepted that evergreen species are dominant in low-nutrient environments and that the evergreen leaf habit represents, to some extent, an adaptation to infertile environments.

These four hypotheses have been developed in specific environmental contexts. The implications of leaf longevity for plant adaptation to its environment must therefore be interpreted in relation to the factor(s) limiting plant growth in a specific environment. In other words, the adaptive significance of leaf longevity is related to the external factor(s) that are the main selecting force for the evolution of leaf traits.

Within the scope of this thesis, my interest focused on the trade-off between leaf longevity and plant adaptation to environments where N-availability for plant growth is low.

1.3 Scaling up from leaf longevity to leaf and plant traits

1.3.1 Leaf longevity and other leaf traits

Reich *et al.* (1997) investigated the relationship in the field between longevity, specific leaf area (SLA), nitrogen content and net photosynthetic capacity (A_{\max}) for leaves belonging to 111 species of trees, shrubs and herbaceous C_3 plants sampled across six diverse biomes. They demonstrated an inverse correlation between leaf life span on one hand, and either mass-based A_{\max} , SLA or mass-based N concentration on the other hand. Slopes of regression did not differ significantly between biomes. In addition, for a given set of 3 leaf traits (e.g. A_{\max} , SLA and leaf nitrogen concentration) they showed a segregation between different plant functional groups: herbs, pioneers, broad-leaved deciduous, broad-leaved evergreen and needle-leaved evergreen. The authors therefore suggested that different plant functional groups maintained constant differences in leaf traits across all biomes. The authors further consolidate their findings by testing and confirming the same relationships on data compiled from the literature for species from several continents and an equally broad array of biomes. This paper is based on a large data set. It provides striking evidence that leaf longevity and, therefore, leaf habit correlate with other leaf traits. The physiological mechanisms involved in plant adaptation to low-nutrient environments might, therefore, be best investigated in relation to a set of leaf traits rather than solely in relation to leaf longevity.

1.3.2 Scaling up from leaf to whole plant

Cornelissen *et al.* (1998) taking a similar approach to that of Reich *et al.* (1997), demonstrated significant differences between seedling mean relative growth rate (RGR) of different life-forms. Deciduous species had a consistently higher RGR than evergreen species. Their findings were based upon experimental studies run under a controlled environment, and provided evidence that RGR is positively correlated with:

- LAR i.e. Leaf area ratio (the ratio of total leaf area and plant dry weight, i.e. indicating allocation at the whole plant level of biomass to leaf area)
- SLA (Cornelissen *et al.*, 1996)

- Mass-based leaf nitrogen concentration (Cornelissen *et al.* 1997).

These studies were restricted to woody species. However, Poorter *et al.* (1990a, 1990b) and Hunt *et al.* (1997) showed that the same relationships between leaf traits and seedling RGR were true for herbaceous species.

Cornelissen *et al.* (1998) compared the values of RGR measured in their study for young seedlings (emergence of first leaf) with, on the one hand, that of older seedlings (over eight weeks old) and on the other hand, with the long-term growth constant (G) calculated for mature trees (Brzeziecki and Kienast, 1994 as cited by Cornelissen *et al.*, 1998). The ranking between species was consistent in both cases, suggesting that fast growing seedlings are produced by fast growing adult trees. These findings together imply that a given set of leaf traits correlates with whole plant growth rate. More specifically, they suggest that deciduous species are fast-growing compared to evergreen species.

1.4 The advantage of being evergreen in low nitrogen environments

Leaf longevity is inversely correlated with SLA, LAR and N concentration, and with plant growth rate. The evergreen and deciduous habits, therefore, involve a suit of functional and structural traits at the leaf and at the whole plant level which might contribute to plant ability to grow in infertile soil. What are those traits and how do they enable plant to cope with low nitrogen availability?

1.4.1 *Slow rates of growth*

At any given time, there is a higher probability that enough nitrogen is available to support the production of a small (slow growth rate) rather than a large biomass (fast growth rate). This is clearly the case for slower growing evergreen, compared to faster-growing, deciduous species (see section above).

1.4.2 *High Nitrogen Use efficiency (NUE)*

1.1.1.1 *At the leaf level*

It has been suggested that evergreen leaves fix more carbon per unit of nitrogen compared to deciduous leaves, due to their longer life-span and the longer average time during which nitrogen is retained by the leaves (Chapin, 1980). This hypothesis is based upon the field study of Small (1972)

relating photosynthetic rates and leaf nitrogen contents of evergreen versus deciduous species growing in infertile (bog) or mesic (non-bog) environments. Small calculated the maximum amount of photosynthate produced by the leaves during their average life span, and found this value to be 235 % higher for the evergreen bog species than for the deciduous ones. However, his calculations assume a photosynthetic activity at its maximal potential throughout the life-span of the leaf, and neglect a potential decrease in photosynthetic capacity as the leaf ages, or a variation in rate of photosynthesis due to environmental factors (i.e. low temperature in winter). Karlsson (1985) compared the leaf carbon gain of a deciduous and an evergreen shrub species. He took into account the decrease through age of photosynthetic capacity and the variations in net C assimilation with light and temperature throughout the growing season. He found there was no difference in the total amount of C gain during the life of the leaf between the two species. Karlsson did not take account of the variations in the amount of nitrogen present in the leaf throughout its life. But his findings clearly suggest that the proposed more efficient use of nutrient by evergreen species (e.g. Chapin, 1980) - as opposed to deciduous species - can not be due to a greater C assimilation per unit nutrient as a consequence of higher leaf longevity.

Chapin and Shaver (1989) defined nutrient use efficiency (NUE) as the inverse of nutrient concentration in the leaves. They compared NUE for nitrogen and phosphorus for arctic species of different growth forms and leaf habits and found a higher NUE for evergreen species compared to deciduous species. It could be argued that NUE, according to their definition, does not provide any information about the efficiency of the use of nitrogen by the plant since it simply reflects variation in leaf nitrogen concentration. Furthermore, the use of nitrogen might be affected by differences in dry matter or nitrogen allocations at the whole plant level, which are not accounted for by studies at the leaf level.

1.1.1.2 *At the whole plant level*

Vitousek (1982) developed the concept of Nutrient Use Efficiency at the whole plant level. He defined it as the “amount of organic matter lost or permanently stored in the plant per unit of nutrient lost or permanently stored in the plant”. According to his definition, a partial index of Nitrogen use efficiency (NUE) is the inverse of nitrogen concentration in the leaf litter. He compared indices of NUE for several

forest stands, and related them to nitrogen circulation, i.e. the total amount of nitrogen lost in litterfall (Vitousek, 1982) or mean annual rates of nitrogen mineralisation (Birk and Vitousek, 1986). These studies seem to be based on the assumptions that nitrogen circulation reflects nitrogen availability for plant uptake and / or mineral nitrogen is the main source of soil N for tree growth. The results suggest that NUE is higher under low nitrogen availability and higher for coniferous (evergreen) forests than temperate deciduous ones. This approach was a major step forward in understanding NUE at the whole plant level and at the community level. However, the assumption that nitrogen circulation reflects nitrogen availability is most secure if comparisons are made between stands of the same developmental stage. This was roughly the case in Vitousek's study since all stands were over 20 years old and no longer showing any substantial increase in the biomass of leaves and twigs. Such an approach restricts the range of communities that can be included in comparative studies. For example, it might not be justified to compare NUE, *sensu* Vitousek, from communities in different successional stages. The assumption that mineral nitrogen represents the main source of soil N is now challenged, as the ability to access organic N has been shown for several species (e.g. Kielland, 1994; Näsholm *et al.*, 1998). In addition, Vitousek's approach did not account for belowground losses of organic matter and nitrogen. The extent to which indices of NUE measured belowground would differ from those measured aboveground is unclear, because there are still very few data in the literature available on the amount of dry matter and nitrogen lost through root turnover. There is substantial evidence that rhizodeposition (i.e. the total amount of carbon lost to the soil) can represent a major sink for carbon (Grayston *et al.* 1996). For example, in Douglas-fir the loss of mycorrhizal fine roots returns two to five time more organic matter to the soil than the turnover of aerial biomass (Fogel and Hunt, 1983). It thus appears necessary to account for belowground as well as aboveground aspects of NUE in order to draw any reliable conclusions about nitrogen use efficiency at the whole plant or community level.

Berendse and Aerts (1987) studied NUE following the same conceptual definition used by Vitousek (1982), but they distinguished two components of NUE:

- Nitrogen productivity (NP = amount of biomass produced per unit nitrogen taken up per unit time)

- Mean residence time of nitrogen (MRT = the average period over which a given unit of nitrogen remains in the plant).

NUE *sensu* Berendse and Aerts is calculated as the product of these two components. Aerts (1990) compared aboveground and whole plant (i.e. including belowground) NUE for two evergreen woody shrubs (*Calluna vulgaris* and *Erica tetralix*) and a perennial deciduous herb (*Molinia caerulea*). The three species had a similar value of NUE when calculated on a whole plant basis. However, the value for the herb was twice that calculated for the woody shrubs when accounting only for aboveground growth. This study demonstrates clearly that NUE is affected by processes happening at the whole plant level, including the belowground part. Furthermore, it gives no support to the suggestion that NUE at the plant level differs between evergreen and deciduous species.

1.4.3 **Mean residence time of nitrogen**

The same study (Aerts, 1990) showed a higher nitrogen productivity (see above) and a lower mean residence time (MRT) of nitrogen for the deciduous herb compared to the evergreen woody shrubs. Using the results of this study and that of three other complementary studies on the same species (Aerts, 1990; Aerts *et al*, 1989; 1991; Aerts and Berendse, 1989), Aerts and van der Peijl (1993) developed a model comparing the equilibrium biomass of each species, i.e. the amount of biomass reached when production equals losses. The output of this model indicated that for a given level of nitrogen availability, a “nitrogen-conserving” species (i.e. evergreen woody shrub with high MRT and low nitrogen productivity) reached a higher equilibrium biomass than a “high-productive” species (i.e. deciduous herb with low MRT and high nitrogen productivity). This suggested that, in the long term, the woody evergreen shrub would outcompete the deciduous grass. Consequently, Aerts (1995) proposed that dominance of evergreen species in nitrogen-poor environments is explained by a high MRT of nitrogen in these species, but his argument was flawed for two major reasons:

1. He compared evergreen with deciduous species of different growth forms (i.e. woody shrub versus herb). On the one hand, the conclusions drawn from the experimental (Aerts and Berendse, 1989; Aerts *et al*, 1990, Aerts, 1990) and the modelling (Aerts and van der Peijl, 1993) studies highlighted the differences between leaf habit as proposed by the author. On the other hand, they could equally

well highlight differences between growth forms. Indeed a given set of leaf traits including leaf longevity correlate with other features which allow different functional groups of plants to be identified, including herbs, deciduous and evergreen woody species (Reich *et al.*, 1997, see section I.3). The different extents to which species in each of these functional groups are adapted to poor-nitrogen environment is then likely to depend upon several different physiological traits, and can not only be attributed to differences in leaf longevity or leaf habit. To understand the adaptive significance of evergreenness, it would be necessary to compare species of contrasting leaf habit within life forms before developing inter-specific comparisons across life forms.

2. Aerts proposed to explain the dominance of evergreen species in nitrogen-poor environments by their high MRT of nitrogen relying upon the outcomes of the Aerts and Van der Peijl (1983) model. Yet it can be argued that this model was based upon three unrealistic major assumptions:
 - Growth was “nutrient-driven” i.e. biomass production was coupled with nitrogen acquisition. This implies that growth can not be sustained by remobilisation of nitrogen stored within the plant.
 - The authors assessed the efficiency of nitrogen uptake by measuring mineral nitrogen availability (Aerts *et al.* 1991). By doing so, it is assumed that uptake of organic N (direct or through mycorrhizal infection) did not occur or contribute to the same extent to N nutrition of all species under study.
 - Steady-state nitrogen content (i.e. nutrient uptake equals nutrient loss) was assumed to calculate MRT and nitrogen productivity. This is not the case for juvenile plants i.e. at the early stage of community establishment and therefore not applicable throughout the growth-period covered by the model. A better estimation of MRT can be achieved using stable isotope techniques, where the fate of a known amount of tracer (e.g. ^{15}N) is monitored (de Aldana and Berendse, 1997). Eckstein *et al.* (1999) compared values of MRT measured through tracer technique or through growth analysis assuming steady-state nitrogen content. Although the highest value of MRT was found among woody evergreen species, there was no significant

difference between MRT for deciduous and evergreen woody species. However, relatively few data were available for comparisons.

Nevertheless, Aerts' proposal represents a useful conceptual framework to understand the adaptive significance of evergreenness in nitrogen -poor habitats, because it highlights the need to distinguish between plant traits associated with nitrogen productivity and those associated with MRT of nitrogen. Aerts (1999) further suggested that high MRT, due to low rates of nitrogen loss, would correlate with low rate of litter decomposition and lead to low rates of mineralisation, which would suggest a positive feed back between the evergreen habit and low nitrogen availability (Aerts, 1999). Hence according to this author, the advantage of being evergreen rather than deciduous in low nitrogen environments is to minimise nitrogen losses from the plants, i.e. increase N conservation within the plant.

Low rates of nitrogen losses can be achieved by low rates of biomass turnover, low nitrogen concentrations, low rates of leaching and / or a high efficiency of nitrogen resorption from senescing tissues. Several studies have assessed one or more of these traits in evergreen and deciduous species (e.g. Chapin and Kedrowski, 1983; Gray, 1983; Aerts, 1990; Shaver and Chapin, 1991; Aerts, 1995; Killingbeck; 1996). From these studies, it is difficult to establish a general trend comparing these traits in species with different leaf habits, because (1) different methodologies were used in the different studies and (2) measurements were often not integrated at the level of the whole plant.

In N-limited environments, one advantage of conserving N within the plant is to reduce competition with other species for N acquisition and ultimately reduce availability of N to other species. However, the conservation of N within the plant might not contribute to plant fitness if N were immobilised in the biomass without contributing to plant growth and / or survival though being recycled. Thus understanding the potential advantages of being evergreen in nitrogen-poor environment should include that the differences exhibited by evergreen and deciduous species in their internal cycling of N.

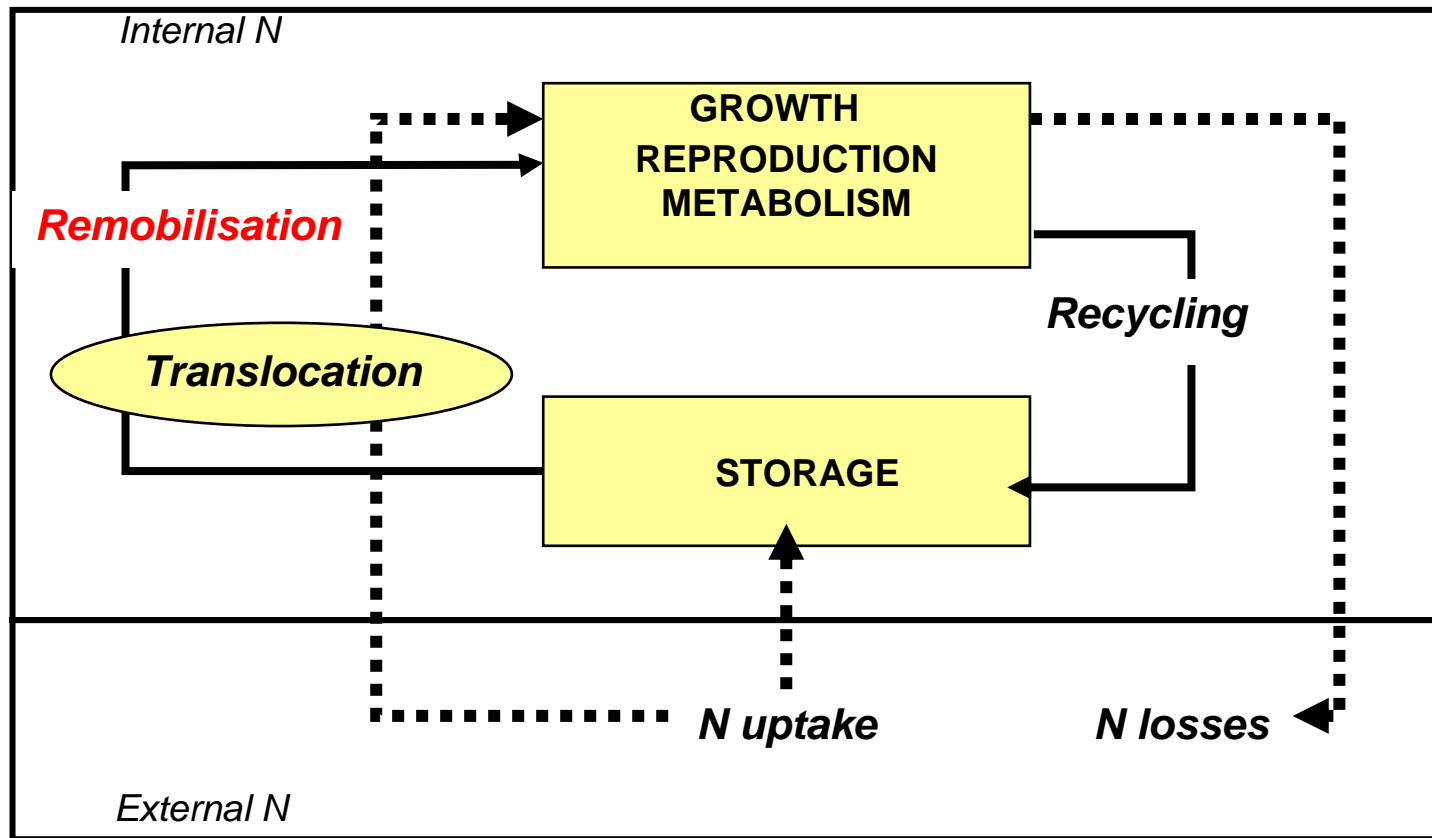


Figure 1.1: Theoretical representation of the internal cycling of nitrogen

II The internal cycling of nitrogen

II.1 Definitions

The internal cycling of nitrogen is the sum of processes involved in the allocation of nitrogen to vegetative and reproductive growth, maintenance, defence and storage (Fig. 1.1).

Chapin *et al.* (1990) defined **growth** as the “build up of those components of biomass that *directly* promote further acquisition and transport of resources.” According to their definition, growth is the synthesis of biomass including structures, biochemical machinery and small pools of metabolic intermediates that contribute to carbon, water and mineral nutrients acquisition and transport.

Maintenance is the sum of metabolic pathways involved in the survival of tissues. It “includes the processes which maintain cellular structures and gradients of ions and metabolites, and also the processes of physiological adaptation that maintain cells as active units in a changing environment” (Penning de Vries, 1975).

Defence is the build up of components involved in deterrence of herbivores and pathogenic organisms (e.g. bacteria or fungi).

Chapin *et al.* (1990) defined **storage** as “resources that build up in the plant and can be mobilised in the future to support biosynthesis for growth or other plant functions.” Their definition is applicable to all resources that plants might store, i.e. water, mineral nutrients and carbon. Millard (1988) proposed that a given resource is “stored if it can be mobilised from one tissue and subsequently reused for the growth or maintenance and defence of another”. This definition is applicable only to mineral nutrients such as nitrogen and phosphorus, as carbon, when considered for its energetic rather than structural value, can be stored and reused in the same tissue. However, the definition of Millard (1988) is more precise and more practical than that of Chapin *et al.* (1990) with respect to demonstrating or identifying storage of nitrogen experimentally.

There are three types of storage (Chapin *et al.*, 1990):

- Accumulation (when supply of resource exceeds demand, *sensu*, Millard, 1988)

- Reserve formation (which occurs even when plant is N deficient)
- Recycling (the mobilisation to storage of N compounds previously used for other plant functions) e.g. the reallocation to storage of N withdrawn during leaf senescence.

Both accumulation and reserve formation lead to increases of N content in the plant, but the chemical forms of N involved in each of these processes can differ. Some forms of N accumulation are immobilised in plant biomass and can not contribute to the internal cycling of N. Thus, it is necessary to demonstrate subsequent remobilisation of N in order to assess storage. Stored N comes either from direct uptake (through reserve formation or accumulation) or from recycling (Fig. 1.1). N requirements for growth, maintenance and defence are met either by remobilisation of N from storage or by direct root uptake. The extent to which a plant can (1) recycle its nitrogen and (2) rely upon stored N or direct uptake, determine both its rate of N losses and its nitrogen use efficiency and thereby contributes to its fitness in nitrogen-poor environments.

11.2 **Ecological significance**

Chapin *et al.* (1990) identified three major advantages of storage:

- To enable plants to recover from catastrophe such as grazing or fire.
- To support reproduction, especially at the end of plant's life (e.g. monocarpic species).
- To allow for asynchrony of supply and demand, i.e. when external availability of N is low compared to plant requirement for N.

This last advantage is particularly important for species growing under temperate climates with a short growing season where spring growth often occurs before rapid N uptake is possible (see section III).

Nambiar and Fife (1987) suggested that the internal cycling of N “enhances the nutrient supply to apical growing points, especially during periods of flushing”; while Jaeger and Monson (1992) suggested that it allows “high demand of simultaneous vegetative and reproductive growth” to be satisfied, and Heilmeier *et al.* (1986) consider that it could allow “species to effectively compete for space and light during the early growing season”. Hence it enables a plant's instantaneous requirement for N to be met

regardless of the current external N availability. Millard (1996) proposed that the ecological significance of storage, and thus that of the internal cycling of nitrogen, is to uncouple growth from N acquisition.

11.3 Assessing the contribution of internal cycling of nitrogen to plant nutrient requirement

Two general approaches have been taken to quantify the internal cycling of N and assess the extent to which it contributes to plant demand:

1. Determination of the efficiency of N retranslocation from senescing tissues, with emphasis on tissues of high turnover rate such as leaves and roots
2. Measurement of the proportion of plant N derived from internal (i.e. storage) or external (i.e. uptake) sources of N

The first approach has focused mainly on N retranslocation from senescing leaves with very few studies attempting to measure N retranslocation from senescing roots (but see Coutts and Philipson, 1976; Ferrier and Alexander, 1991; Nambiar and Fife, 1987, 1991; Aerts, 1990). There is not enough information about N retranslocation from roots to recognise any general pattern relating to plants sharing the same leaf habit or belonging to the same growth form. There have been numerous studies assessing N retranslocation from leaves and these have been reviewed by several authors (e.g. Aerts, 1996; Killingbeck, 1996). However, a clear understanding of the factors controlling N withdrawal from leaves has been limited because of inconsistencies in the approaches taken by different authors such as the use of differences in mass-based rather than area-based leaf N concentration to assess the efficiency of N retranslocation before abscission, thereby ignoring possible retranslocation of other compounds such as carbohydrates.

The first approach, restricting that the internal cycling of N to N withdrawal from senescing tissues, was misleading because it ignored any recycling of N from sites of storage to sites of N use (new vegetative growth, reproduction, defence or maintenance) outside the context of senescence.

The second approach has used N budgets to quantify the internal cycling of N. At the stand level, budgets have been based on the measurement of (i) N distribution between the different components within the stand, such as crop standing parts (foliage, stem wood, branches bark or roots), litter or soil N, and (ii) fluxes of N between these components (through fall, N release by litter fall decomposition) plus N output and input to the stand (substrate leaching or N deposition through rainfall). Such studies have allowed the N requirement of crops over a defined period of time to be quantified and compared with the potential contribution of root uptake to crop N, thereby providing an estimation of the amount of N withdrawn from leaves before senescence (retranslocation) and recycled within the crop (e.g. Miller, 1984, 1986). At the whole plant level, budget studies have measured seasonal variations of N contents in different components of the plant (e.g. foliage, stems, wood or bark, fine or woody roots) using sequential destructive harvests. The contribution of N remobilisation through internal cycling was then assessed by comparing the amount of N incorporated into newly formed biomass to corresponding decreases of N content in previously formed tissues (e.g. Jaeger and Monson, 1992). Budget studies assume that the amount of N recycled is equal to that withdrawn from senescing tissues (e.g. Meier *et al.*, 1985) thereby ignoring N recycled within the plant independently of senescence. Alternatively, stand budget studies estimated the contribution of internal N to stand production by subtracting N taken up from the total amount of N required by the standing crop (e.g. Helmisaari, 1995). Often the amount of N taken up was assumed to be that available in the soil as mineral N. Thus it ignored the possibility of N taken up in its organic form. Whole plant budget studies are relatively imprecise because of the errors associated with sub-sampling mature trees, and a failure to complete budgets by ignoring both belowground N distribution and fluxes and withdrawal of N from leaves independently of senescence (Millard, 1996).

In other studies, the internal cycling of N has been assessed using tracer techniques with time-differential levels of stable isotope (^{15}N) enrichment. These techniques allow a distinction to be made between N acquired in previous periods (i.e. N remobilised) and N currently acquired. Even so, field studies can still be approximate because accounting for endogenous soil ^{15}N will depend on soil factors and the type, amount and enrichment of fertiliser N applied. To eliminate errors associated with uncontrolled variations in soil endogenous ^{15}N , tracer techniques have been applied to plants grown in

sand culture. These studies have provided strong evidence that the internal cycling of N contributes an important proportion of N required for growth or reproduction (Table 1.1). Furthermore, these studies have allowed certain features of the internal cycling of N to be clarified:

- The amount of N remobilised depends on N supply at the time when storage N was built up but does not depend on N current supply (e.g. Millard and Proe, 1993).
- The magnitude of N uptake depends on the external supply of N so the relative contribution of remobilised versus N uptake depends on the time when the measurement is made, and on external N supply (e.g. Millard and Proe, 1993; Fouldrin and Limami, 1993; Deng *et al.*, 1989).

Table 1.1. The percentage of new growth N derived from remobilisation

Species	growth form	Leaf habit	Stage plant /stand ¹	Time scale of measurement ²	Reference	Percent remobilised N
<u>Seasonal/ spring growth</u>						
<i>Polygonum bistortum</i> L.	herb	Deciduous	Mature wild plant	1 GS (2-3 months)	4	60
<i>Rhododendron lapponicum</i> L.	Shrub	Evergreen	Mature wild plant	1 GS (4 months)	5	77
<i>Betula</i> species	Tree	Deciduous	45 yr-old, felling	45 year-rotation	6	47
<i>Juglans regia</i> L.	Tree	Deciduous	Mature	1 month aBB	7	90-95
<i>Juglans regia</i> L.	Tree	Deciduous	Mature	2 months aBB	7	65-80
<i>Pinus</i> species	Tree	Evergreen	10 yr-old, felling	1 year aBB	8	17
<i>Pinus</i> species	Tree	Evergreen	40 yr-old, felling	1 year aBB	8	50
<i>Malus domestica</i> Borkh.	Tree	Deciduous	3 yr-old GT	2 months aBB	9	52-87
<i>Ulex europaeus</i> L.	Shrub	Evergreen	2 yr-old RC	2 months	10	30-52
<i>Vaccinium ashei</i> Reade	Shrub	Deciduous	2 yr-old S	1 month aBB	11	66-92
<i>Vaccinium ashei</i> Reade	Shrub	Deciduous	2 yr-old S	3 months aBB	11	18-45
<i>Betula pendula</i> Roth.	Tree	Deciduous	4 yr-old S	1.5 months aBB	12	51

¹ S=saplings or seedlings; GT=grafted trees; RC=rooted cuttings; Mrc=minisward regularly clipped

² aBB=after budbreak; aL=after the start of the labelling period; GS=growing season

Table 1.1. (continued)

Species	growth form	Leaf habit	Stage plant /stand ³	Time scale of measurement ⁴	Reference	Percent remobilised N
<i>Betula pendula</i> Roth.	Tree	Deciduous	4 yr-old S	5 months aBB	12	41
<i>Picea sitchensis</i> (Bong.) Carr.	Tree	Evergreen	5 yr-old S	1 month aBB	13	70
<i>Picea sitchensis</i> (Bong.) Carr.	Tree	Evergreen	5 yr-old S	1 months aBB	13	90
<i>Picea sitchensis</i> (Bong.) Carr.	Tree	Evergreen	5 yr-old S	1 months aBB	13	36
<i>Picea sitchensis</i> (Bong.) Carr.	Tree	Evergreen	5 yr-old S	1 months aBB	13	76
<i>Pinus sylvestris</i> L.	Tree	Evergreen	4 yr-old S	1.5 months aBB	12	47
<i>Pinus sylvestris</i> L.	Tree	Evergreen	4 yr-old S	5 months aBB	12	30
<i>Prunus persica</i> L.	Tree	Deciduous	3 yr-old GT	1 months aBB	14	93
<i>Prunus persica</i> L.	Tree	Deciduous	4 yr-old S	2 months aBB	14	68
<i>Pyrus communis</i> L.	Tree	Deciduous	2 yr-old GT	2 months aBB	15	55-60
<i>Sorbus acuparia</i> L.	Tree	Deciduous	4 yr-old S	1.5 months aBB	12	80
<i>Sorbus acuparia</i> L.	Tree	Deciduous	4 yr-old S	5 months aBB	12	32

³ S=saplings or seedlings; GT=grafted trees; RC=rooted cuttings; Mrc=minisward regularly clipped

⁴ aBB=after budbreak; aL=after the start of the labelling period; GS=growing season

Table 1.1. (continued)

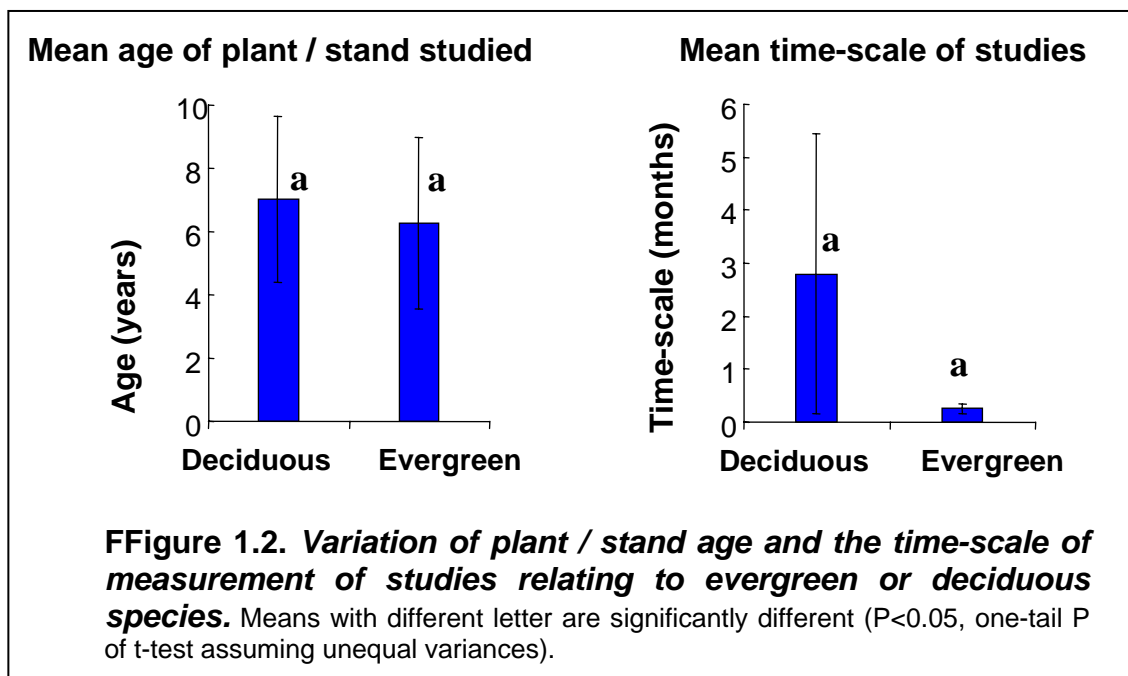
Species	growth form	Leaf habit	Stage plant /stand ⁵	Time scale of measurement ⁶	Reference ⁷	Percent remobilised N
<u>Regrowth after forcing</u>						
<i>Chicorium intybus</i> L.	herb	Deciduous	1 yr-old tuber	3 weeks aBB	1	70
<i>Chicorium intybus</i> L.	herb	Deciduous	1 yr-old tuber	3 weeks aBB	1	90
<u>Regrowth after defoliation</u>						
<i>Agrostis castellana</i> L.	herb	Deciduous	3 month-old Mrc	1 month aL	2	59
<i>Festuca rubra</i> L.	herb	Evergreen	3 month-old Mrc	1 month aL	2	65
<i>Lolium perenne</i> L.	herb	Evergreen	2 month-old Mrc	3 weeks aL	3	25-35
<i>Lolium perenne</i> L.	herb	Evergreen	3 month-old Mrc	1 month aL	2	39
<i>Poa trivialis</i> L.	herb	Evergreen	3 month-old Mrc	1 month aL	2	46

⁵ S=saplings or seedlings; GT=grafted trees; RC=rooted cuttings; Mrc=minisward regularly clipped

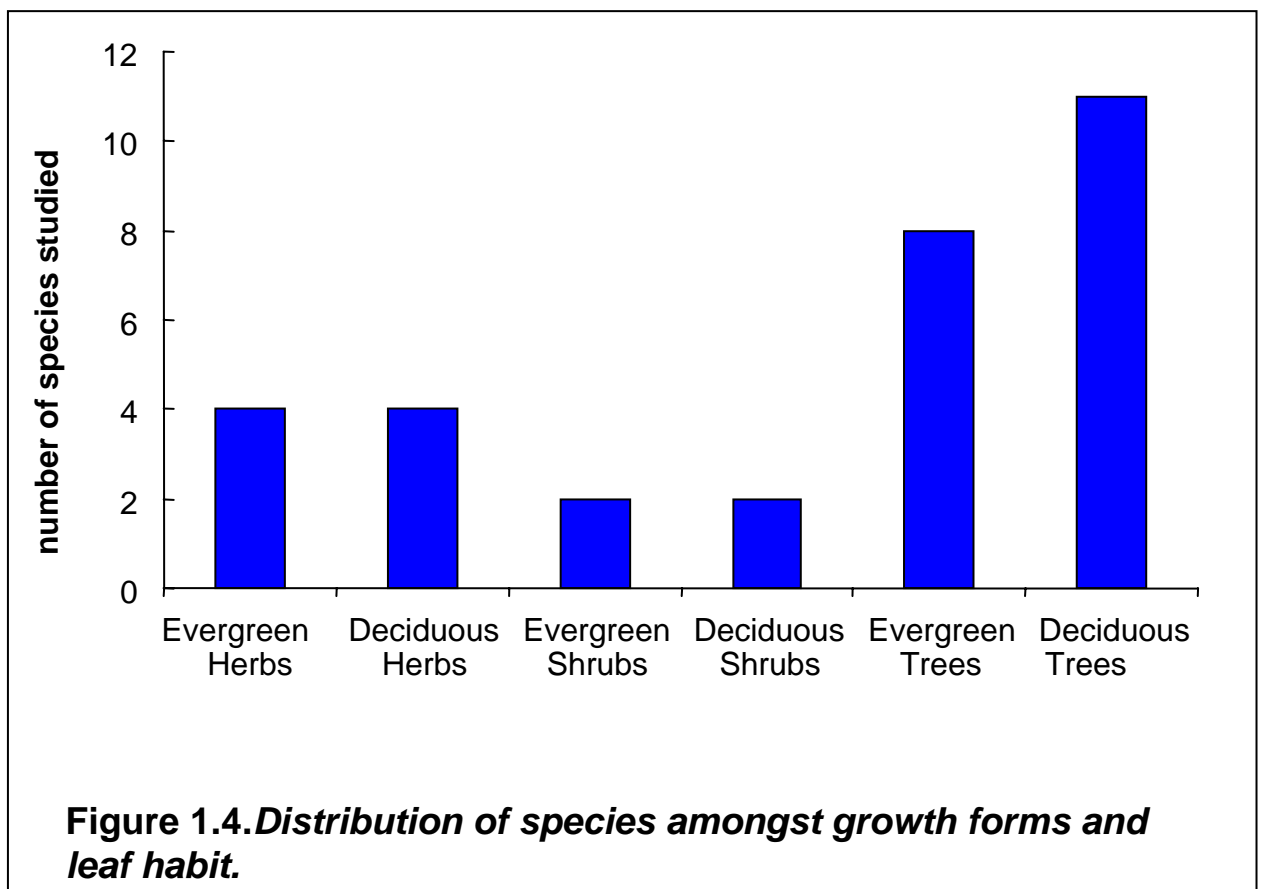
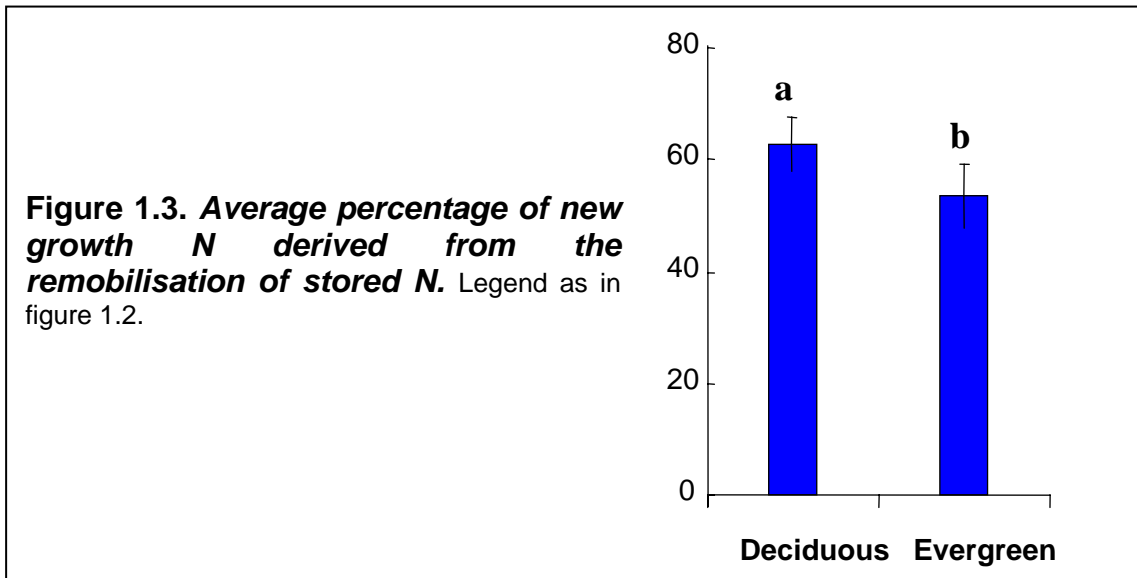
⁶ aBB=after budbreak; aL=after the start of the labelling period; GS=growing season

⁷ 1=Fouldrin and Limami, 1993; 2=Thornton et al., 1994; 3=Thornton et al., 1993; 4=Jaeger and Monson, 1992; 5=Karlsson, 1994; 6=Miller, 1984; 7=Deng et al., 1989; 8=Miller, 1986; 9=Neilsen et al., 1997; 10=Thornton et al., 1995; 11=Birkhold and Darnell, 1993; 12=Millard et al., 2001; 13=Millard and Proe, 1993; 14=Munoz et al., 1993; 15=Tagliavini et al., 1997.

The extent to which evergreen or deciduous species depend on N remobilisation can, therefore, only strictly be compared if the data available come from plants of comparable developmental stages and supplied with comparable levels of N. In Table 1.1. the range of variation in age of plant / stand or in the time-scale of measurement is similar for evergreen and deciduous species (Fig. 1.2).



On average, deciduous species rely more on their internal cycling of N to meet their N requirement for growth compared to evergreen species, with 63 % and 54 % of new growth N derived from remobilisation in deciduous and evergreen species, respectively (Fig. 1.3). However, the figures reported in this review are biased towards trees (Fig. 1.4) and formed a relatively small data set (only 19 species), therefore any trends apparent from this study need to be validated on a larger data set representative of all growth forms (i.e. trees, herbs and shrubs).



The results of the analysis of this small data do not support the idea that evergreen species rely more upon N internal cycling than deciduous species. However, leaf habit might also affect other aspects of the internal cycling of N, such as sites of storage or the time-course of N remobilisation, which might affect plant growth pattern in a manner favouring evergreenness in N limiting conditions.

Table 1.2. Sites of storage and characteristics of the dynamic of N remobilisation in a wide range of species

Plant details				Methodology			Data			
1. Species	2. Growth Form ⁸	3. Leaf habit ⁹	4. Climate ¹⁰	5. Stage plants ¹¹	1. Type of study ¹²	2. Direct evidence of remobilisation	1. Sites of N storage	2. Timing N fluxes in new growth	3. Time-length remobilisation (months)	References ¹³
<u>Spring growth</u>										
<i>Daucus carota</i> L.	BH	D	T	Fied-grown S	F	no	Hypocotyl-tap root			22
<i>Arctium tomentosum</i> Mill.	BH	E	T	Fied-grown S	F	no	Hypocotyl- tap root			22
<i>Cirsium vulgare</i> Savi.	BH	E	T	Fied-grown S	F	no	Hypocotyl-tap root			22
<i>Dipsacus sylvester</i> Huds.	BH	E	T	Fied-grown S	F	no	Hypocotyl-tap root			22
<i>Molinia caerulea</i> L. (Moench)	PH	D	T	Plants collected from field	S	yes	Roots-basal internodes	remobilisation before uptake	2.5	25
<i>Polygonum bistortum</i> L.	PH	D	A	Adult wild plants	F	no	Rhizome	coincident		3, 4
<i>Rhododendron lapponicum</i> L.	S	D	SA	>3 yr-old branches	F	no	previous year leaves		2.5	5
<i>Vaccinium ashei</i> L.	S	D	T	1-yr old S	P	yes	Roots, stems	remobilisation before uptake		1
<i>Ulex europaeus</i> L.	S	E	M	2 yr-old RC	S	yes	Stems/spines-roots			27
<i>Acer freemanii</i> E. Murr	T	D	T	2 yr-old RC	P	no	Roots (Stems?)			19

⁸ BH = Biennial herb; AH = Annual herb; PH = Perennial herb; S = Shrub; T = Tree

⁹ D = Deciduous ; E = Evergreen (for herbaceous plants are classified as Deciduous / Evergreen if they dye back / persist in the winter.

¹⁰ T = Temperate; M = Mediterranean; A = Alpine; SA = Subarctic; ST = Subtropical

¹¹ S =seedlings or saplings; RC = rooted cuttings; GT = grafted tree ; M = minisward

¹² F= field ; P = pot culture in soil; S= pot culture in sand ¹³ see end of table

Table 1.2. (continued)

Plant characteristics					Methodology		Data			Ref	
1	2	3	4	5	1	2	1	2	3		
<i>Acer pseudoplatanus</i> L.	T	D	M	2 yr-old S	S	yes	Roots (2/3rd)-Stems (1/3rd)				13
<i>Acer pseudoplatanus</i> L.	T	D	M	8 yr-old trees	F	yes		remobilisation before uptake			17
<i>Betula pendula</i> Roth.	T	D	T	2 yr-old S	S	yes	Roots (1/2)-Stems (1/2)	remobilisation before uptake	1.5		15
<i>Betula pendula</i> Roth.	T	D	T	4 yr-old S	S	yes	Woody tissues (roots, stems)	coincident	2		16
<i>Eucalyptus globulus</i> Labill.	T	D	M	1 yr-old S	S	no	previous year leaves				29
<i>Fraxinus exclesior</i> L.	T	D	T	10 yr-old trees	F	yes	Stems (bark-wood)				9
<i>Fraxinus exclesior</i> L.	T	D	T	3 yr-old S	S	yes	Roots				9
<i>Malus domestica</i> Borkh.	T	D	T	1 yr-old GT	S	yes	Stems	remobilisation before uptake			11, 12
<i>Pistacia vera</i> L.	T	D	M	20 yr-old-heavy cropping	F	no	Stems (1/3rd)-Roots (2/3th)				20
<i>Pistacia vera</i> L.	T	D	M	20 yr-old-light cropping	F	no	Stems (1/5th)-Roots (4/5th)				20
<i>Prunus persica</i> L.	T	D	T	2 yr-old GT	P	yes	Roots-twigs	remobilisation before uptake	2		23, 24
<i>Pyrus communis</i> L.	T	D	T	5 yr-old GT	F	no	Frame(trunk,2 yr-old branches)				21
<i>Pyrus communis</i> L.	T	D	T	1 yr-old GT	S	yes		remobilisation before uptake	1.5		23
<i>Sorbus aucuparia</i> L.	T	D	T	4 yr-old S	S	yes	Woody tissues (roots, stems)	remobilisation before uptake	1.5		16
<i>Citrus unshiu</i> Marc.	T	E	T	21 yr-old tree	F	no	Woody tissues (trunc, large branches and roots)				6
<i>Picea sitchensis</i> (Bong.) Carr.	T	E	T	4 yr-old S	S	yes	Previous year needles				14

Table 1.2. (continued)

Plant characteristics					Methodology		Data			Ref ¹³	
1	2	3	4	5	1	2	1	2	3		
<i>Pinus radiata</i> D. Don	T	E	M	1 to 4 yr-old S	F	no	Previous year needles- others?				18
<i>Pinus sylvestris</i> L.	T	E	M	4 yr-old S	S	yes	Previous year needles	coincident		2	16
<u>Regrowth after defoliation</u>											
<i>Medicago sativa</i> L.	PH	D	M	nodulated	P	yes	Roots (tap, laterals)-crown stems and leaves				7, 8
<i>Molinia caerulea</i> L. (Moench)	PH	D	T	Plants collected from field	S	yes	Roots-basal internodes				25
<i>Agrostis castellana</i> L.	PH	E	T	3 months-old M	S	yes	all remaining plant parts				26
<i>Festuca rubra</i> L.	PH	E	T	3 months-old M	S	yes	all remaining plant parts				26
<i>Lolium perenne</i> L.	PH	E	T	3 months-old M	S	yes	all remaining plant parts			1	26
<i>Poa trivialis</i> L.	PH	E	T	3 months-old M	S	yes	all remaining plant parts				26
<i>Trifolium repens</i> L.	PH	E	T		P	yes	root-stolon				10
<u>Reproduction</u>											
<i>Helianthus annuus</i> L.	AH	-	M	Cv. Hysun 30	S	yes	All vegetative organs	coincident			2
<i>Zea mays</i> L.	AH	-	ST	Field-grown S	F	yes	all tissues but mostly stalk, leaves	coincident			28
<i>Molinia caerulea</i> L. (Moench)	PH	D	T	Plants collected from field	S	no	Leaves				25

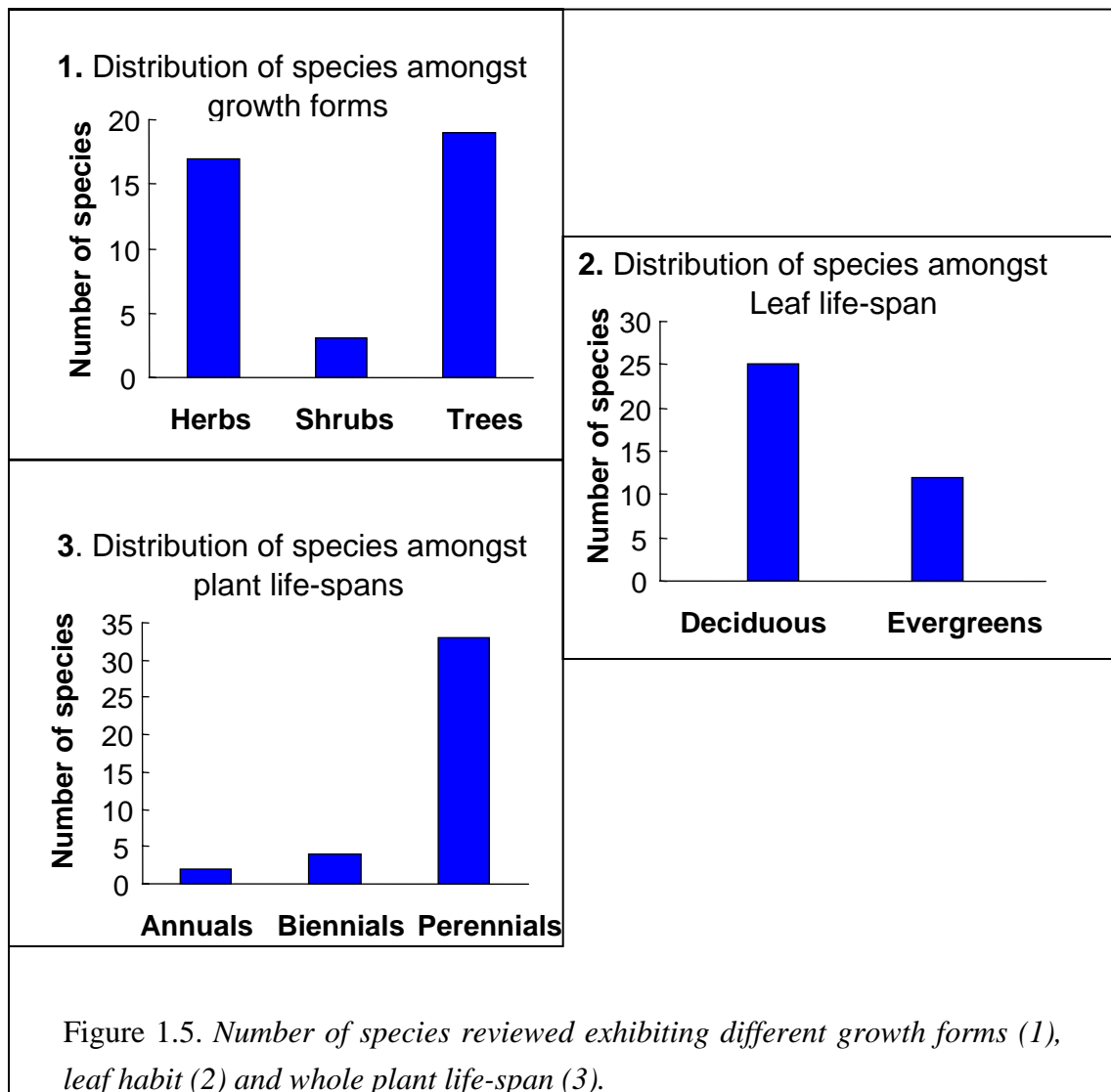
¹³ **1** = Birkhold and Darnell, 1993; **2** = Hocking and Steer, 1995; **3** = Jaeger and Monson, 1992; **4** = Lipson et al., 1996; **5** = Karlsson, 1994; **6** = Kato et al., 1984; **7** = Kim et al., 1993; **8** = Ourry et al., 1994; **9** = Marmann et al., 1997; **10** = Marriott and Haystead, 1990; **11** = Millard and Neilsen, 1989; **12** = Neilsen et al., 1997; **13** = Millard and Proe, 1991; **14** = Millard and Proe, 1993; **15** = Millard et al., 1998; **16** = Millard et al., 2001; **17** = Millard, 1994; **18** = Nambiar and Fife, 1987; **19** = Rose and Biernacka, 1999; **20** = Rosecrance et al., 1998; **21** = Sanchez et al., 1991; **22** = Steinlein et al., 1993; **23** = Tagliavini et al., 1997; **24** = Munoz et al., 1993; **25** = Thornton and Millard, 1993; **26** = Thornton et al., 1993; **27** = Thornton et al., 1995; **28** = Weiland and Ta, 1992; **29** = Wendler et al., 1995.

11.4 Characteristics of the internal cycling of N in relation with leaf habit.

11.4.1 *Sites of storage*

Many studies investigating storage have reported variations over time in concentration of N in different organs or tissues. These studies provide limited data on N storage as increases in concentration could be caused by accumulation of N in chemical forms, which are subsequently not remobilised, or by a change in the concentration of other non-N containing compounds (e.g. carbohydrates). Other studies have measured changes in N amounts, but very few of them have assessed changes in all parts of the plant (Chapin and Kedrowski, 1983), which provide information about the contribution of different organs to N storage. Results from the few studies assessing changes in N content of all the plant parts are presented in Table 1.2. The specific sites of N storage appeared to depend on plant leaf habit or growth form since (1) woody perennials (trees and shrubs), as opposed to herbaceous species, store most of their N in their woody tissues and (2) most evergreen species store N in their leaves, whilst deciduous species store N in roots and / or stems.

However, the information presented in Table 1.2. is largely biased towards deciduous species (as opposed to evergreen species) and towards perennial as opposed to annual and biennial plants (Fig. 1.5, charts 2 and 3). Furthermore, shrub species are very poorly represented (Fig. 1.5, chart 1). The extent to which sites of N storage could differ in species adapted to different type of environments could not be assessed, because few data were available for species originating from rich-N environment other than crops (fruit trees) or from climates others than temperate.



11.4.2 Dynamic of N remobilisation

Very few studies have actually demonstrated the remobilisation of N from sites of storage to sites of N utilisation for growth, maintenance, defence or reproduction. Such demonstration is necessary, as an organ could be exporting stored N and importing N simultaneously. For example, Hocking and Steer (1995) found that remobilised N contributed 1/3rd of mature seed N in sunflower. They found a net decrease in the N content of roots and leaves during the period over which remobilisation occurs, but there was no net variation in the N content of stems. However, using ¹⁵N labelling of N uptake, they found that between 45 % and 95 % of the N taken up at the end of floret initiation and incorporated in stem biomass was remobilised to sustain seed maturation. Therefore, this study demonstrated that variations in N pools should be interpreted with caution or coupled with the use of N labelling (e.g. stable

isotope ^{15}N) to distinguish current uptake of N from N stored within the plant. Table 1.2. shows data from studies using ^{15}N labelling which investigated the dynamics of N remobilisation (timing and time-length). The time-length over which remobilisation occurs, and the relative timing of N fluxes into new growth which are derived from uptake or from remobilisation depend on the species. Further investigation is required to assess the extent to which growth form, leaf habit and possibly climate influence these features of N remobilisation. Millard and Proe (1993, see Table 1.2) demonstrated that the amount of N remobilised in spring to support new growth depended solely on the size of N stores within the trees (*Picea sitchensis* (Bong.) Carr.). In the context of their experiment, this was also true for the rate of remobilisation. Irrespective of current N supply, 3.4 and 0.8 mg N per day were incorporated in the new shoot for plants with High N and low N storage status, respectively. These results suggested that the rate, amount and duration of remobilisation depends on plant storage status, which is in turn affected by N availability at the time of storage build up. In the long term, species adapted to poor N environments might have evolved to exhibit different relationship between external N availability and plant N storage status and / or different relationship between N storage status and patterns of N remobilisation.

II.5 Feedback between the internal cycling of N and plant growth, as related to leaf habit

II.5.1 Implications of different sites of N storage for evergreen and deciduous species

In temperate regions, most stored N is retained overwinter in the leaves of evergreen species, and in the stems and roots of deciduous species. However, irrespective of leaf habit, developing leaves are the strongest sink for N during the spring and summer. Thus, for evergreen species, leaves are both a major sink for N and a main site of storage. N incorporated in leaf biomass during spring and summer can be used both for C acquisition during the growing season and can contribute to increase overwintering N reserves without any retranslocation from the site of use to the site of storage (e.g. Camm, 1993). Alternatively, in evergreen species, leaf sink strength during flushes of growth may enhance accumulation of N in the leaves, which is not correlated with an increase in photosynthetic activity and can, therefore, be interpreted as contributing to the build up of N storage (e.g. Livingston *et al.*, 1998).

For deciduous species, the recycling of N incorporated in leaf biomass over spring and summer is possible only after retranslocation to storage sites – i.e. stems and roots – before leaf abscission. Leaf N can contribute a substantial amount of N remobilised the following spring. Millard and Thomson (1989) calculated that between 32 % and 48 % of N remobilised for apple new spring growth was derived from the recycling of leaf N which has been directly involved in C acquisition during the previous growing season. This suggests that the factors controlling retranslocation of N before leaf abscission in deciduous species can have a direct effect on N internal cycling. If the internal cycling of N is a process enabling the plant to uncouple N use from N acquisition, one might expect N retranslocation during leaf senescence of deciduous species to be greater when external N supply is low. Numerous studies have investigated the effects of N availability on N retranslocation from leaves, and their results are often contradictory (e.g. Millard and Thomson, 1989; Killingbeck, 1996). Therefore the effect of N supply on the amount of N withdrawn from deciduous leaves in autumn and its impact on N internal cycling is unclear. Furthermore, the extent to which recycled leaf N contributes to N storage could be reduced when plants are supplied with generous amount of N in autumn. Tagliavini *et al.* (1999) estimated that the amount of N remobilised to support spring growth of pear originating from N taken up during the previous autumn was twice the amount originating from uptake during the previous spring and summer (including N allocated to leaf growth in spring and summer and withdrawn from leaves before senescence). The timing of N supply, i.e. during leaf growth or during leaf senescence is very likely to have different effect of N internal cycling. In the light of the results reported by these authors, it could be hypothesised that the build up of N storage in autumn relies preferentially on N derived from current root uptake as opposed to N withdrawn from senescing leaves. When autumnal N supply is generous, root uptake is the main source of N for storage build up, whilst more N is withdrawn from senescing leaves and retranslocated to sites of storage when autumnal N supply is poor. If this hypothesis is valid, the proportion of N withdrawn from senescing leaves would depend mostly on N availability at the time of leaf senescence, while the total amount of N which can be withdrawn from senescing leaves would depend on N availability at the time of leaf growth. In deciduous species, the extent to which recycling of leaf N contributes to N storage would depend on N availability both at the time of leaf growth and at the time of leaf senescence. On the

one hand, the fact that deciduous species need to retranslocate leaf N before abscission can be viewed as a disadvantage compared to evergreen species in low-N environment because it implies greater losses of N. On the other hand, it could be viewed as a means to increase plant response to temporal variation in N external availability.

11.5.2 **Factors controlling N remobilisation in relation to leaf habit**

Several studies have demonstrated that N remobilisation in spring is not dependent on the level of current N supply (e.g. Fouldrin and Limami, 1993) but depends upon the amount of N stored the previous year (e.g. Millard and Proe, 1993; Thornton *et al.*, 1995). These results suggest that N remobilisation is a “source-driven” process. In contrast, Nambiar and Fife (1987, 1991) suggested that nutrient remobilisation is driven by shoot growth. These authors therefore implied that N remobilisation is a “sink-driven” process. These interpretations of the factors controlling N remobilisation seem, in the first instance, to be contradictory. Yet, for perennial plants in temperate climates, N stores are built up in late summer and autumn, at the same time as buds are set for growth in the next spring. It could then be hypothesised that the amount of N storage in autumn is balanced to match the initial requirement for N for next year’s growth. N remobilisation could then be driven by both the amount of N in store (source) and shoot growth (sink) as both sink and source strength could be adjusted in the previous year to match each other. For both evergreen and deciduous species, there is indirect evidence of feedback between N source and sink. For *Populus deltoides* (deciduous), the removal of buds i.e. sink in early spring inhibited N remobilisation from storage i.e. source (Coleman *et al.*, 1993). For *Pinus sylvestris* (evergreen), defoliation of year-old needles (source) before budbreak significantly decreased the mass and length of newly grown needles (sink) later on in the season, while defoliation of two year-old needles had no effect on new shoot growth (Honkanen *et al.*, 1999). The differential effect of defoliation of one and two year-old needles can be explained by N being remobilised predominantly from one-year old needles (Millard *et al.*, 2001).

III The ecophysiology of *Vaccinium myrtillus* and *Vaccinium vitis-idaea*

III.1 Ecology

III.1.1 *Distribution*

Vaccinium myrtillus (bilberry) and *Vaccinium vitis-idaea* (lingonberry / cowberry) are widespread over Europe. Their distribution extends from Iceland to northern Norway, Spain and the Caucasus. *V. myrtillus* is also found in north Asia, while *V. vitis-idaea* is found in North America (Welch *et al.*, 1994; Grime *et al.* 1988).

III.1.2 *Habitat*

Both species commonly co-occur in upland pastures, moorlands and heathlands, and in open woodlands and coniferous plantations (e.g. Fig. 1.6). They are also present in peat bogs and montane habitats such as scree and boulder slopes (Clapham *et al.*, 1962). In the British Isles, they can be found between 200 and 1300m above sea level. Both species tolerate shade and burning but are sensitive to water logging. *V. myrtillus*, in particular, is frost-sensitive so its distribution depends on snow protection (Gimingham, 1982; Tolvanen, 1997). However, the habitats of both seem to be determined by edaphic rather than by climatic factors (Ritchie, 1955,1956). They are mainly found on sloping ground (but at less than 60 degrees), on well-drained, acidic soils (preferentially pH<4.5, maximum pH=6.8), with large quantities of persistent litter, and rarely on bare soils.



Figure 1.6. *Vaccinium myrtillus* (Vm) and *Vaccinium vitis-idaea* (Vvi) in the understorey of an open woodland.

Such soils are typically classified as infertile, nitrogen-poor, rich in organic matter. Concentrations of organic N compounds such as “free” amino acids are high (Abuarghub and Read, 1988; Kielland, 1994). Availability of mineral nitrogen is low and follows marked seasonal patterns with peak values in mid-spring and autumn (Morecroft *et al.*, 1992; Williams, 1992). Rates of mineralisation show the same seasonal trends, and altitudinal variations appear to be smaller in magnitude than changes during the

course of the year (Morecroft *et al.*, 1992). When available, mineral nitrogen is considered to be present as ammonium rather than nitrate (Troelstra *et al.*, 1990; Williams, 1992), although there may be rapidly turning over nitrate pools. Hence, species adapted to these habitats, such as *V. myrtillus* and *V. vitis-idaea*, are thought to acquire nitrogen mainly as ammonium or in organic forms.

III.2 **Growth pattern: morphology and phenology**

III.2.1 ***Morphology***

V. myrtillus and *V. vitis-idaea* have similar life forms i.e. both are dwarf shrubs and are perennial woody chamaephytes. They form extensive long-lived clones (30 years, Flower-Ellis, 1971) with rhizomes located 100-200 mm below the soil surface, which explains their tolerance to burning and susceptibility to water logging. Both have the typical ericaceous root system, highly branched, with adventitious fine roots of high specific root length (so-called “hairy” roots) and early suberization and cork covering of primary roots (Heath and Luckwill, 1938).

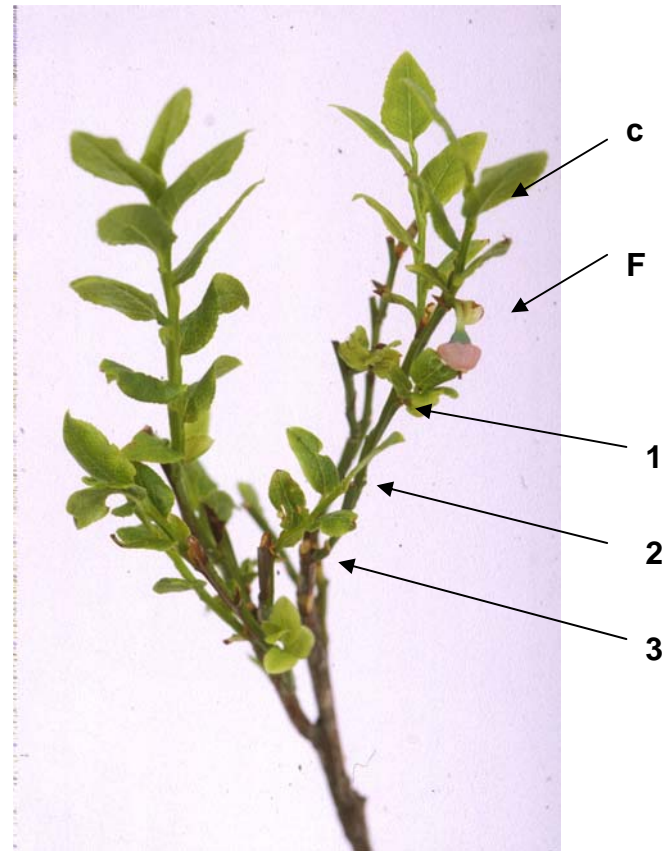
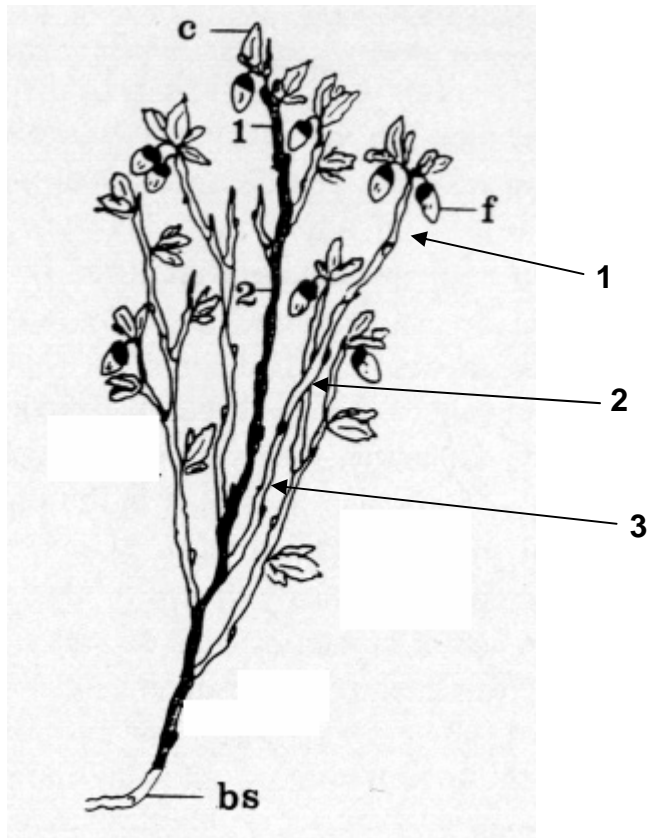


Figure 1.7.a. *Aboveground morphology of Vaccinium myrtillus.* Arrows indicate current year shoot (c) including flower (f), one, two and three year-old shoots (1, 2 and 3, respectively), and belowground bud (bs) on the rhizome. Diagram is reproduced from Tolvanen (1995).

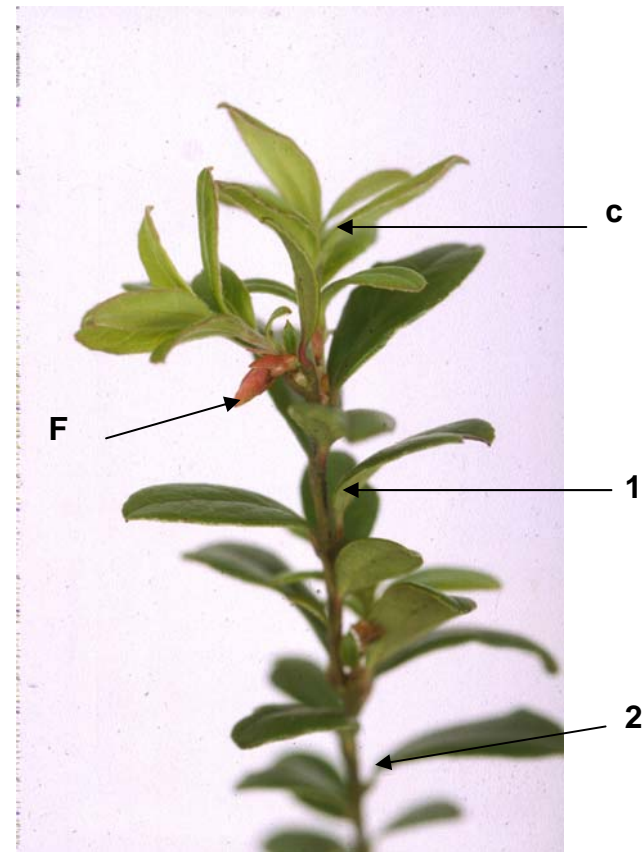
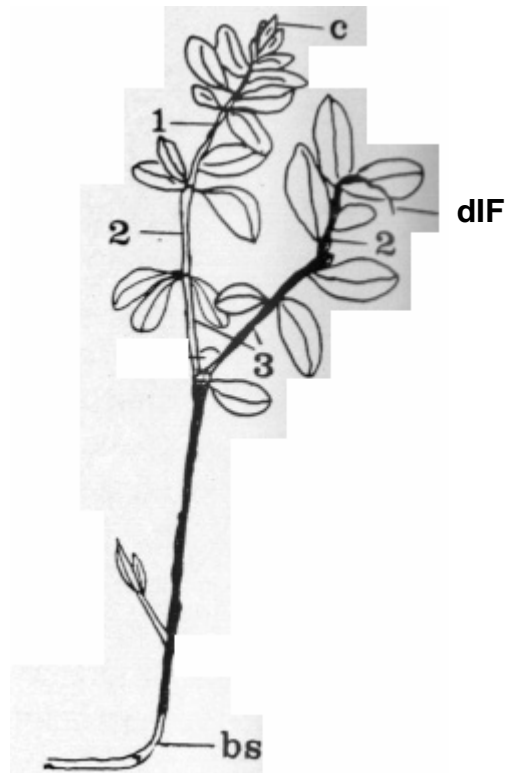


Figure 1.7.b. *Aboveground morphology of Vaccinium vitis-idaea.* Arrows indicate current year shoot (c) including inflorescence (f), one, two and three year-old shoots (1, 2 and 3, respectively), one-year-old inflorescence (dead, dIF) and belowground bud (bs) on the rhizome. Diagram is reproduced from Tolvanen (1995).

Both produce aerial and orthotropic ramets from buds on the horizontal belowground stems i.e. the rhizomes, but their morphology aboveground differs (Fig. 1.7):

- They both have small, broad, leathery leaves, but *V. myrtillus* has short-lived leaves (life-span less than 10 months, Flower-Ellis, 1971) whereas *V. vitis-idaea* has long-lived leaves (life-span between 2.5 and 4 years, Karlsson, 1992).
- While mean area per leaf and mean length of internode have comparable dimensions for both species, stems and leaves are thicker for *V. myrtillus* and *V. vitis-idaea*, respectively.
- Stems are cylindrical for *V. vitis-idaea* but 3-angled for *V. myrtillus*. In the case of *V. myrtillus*, stems remain green for at least one year and are potentially photosynthetic, due to their thickness and angular shape (Welch *et al.*, 1994).
- *V. myrtillus* grows up to 60 cm tall while the maximum shoot height is 30 cm for *V. vitis-idaea*.
- Vegetative growth of *V. myrtillus* is sympodial, highly branched, with weak apical dominance. By contrast, growth of *V. vitis-idaea* is predominantly monopodial, less branched and with stronger apical dominance (Tolvanen, 1995).
- *V. vitis-idaea* produces an inflorescence with up to 10-12 flowers in a cluster, whereas *V. myrtillus* produces one or two flowers from reproductive buds, which are axillary to the apical vegetative bud. Growth of the axis bearing the flowers will cease after fruiting in the case of *V. vitis-idaea*, while it will either continue or die back in the case of *V. myrtillus*.

III.2.2 **Phenology**

There are few data available about belowground growth. Kummerow and Russell (1980) reported an increase in the biomass of roots and rhizomes in spring, starting after budbreak and lasting until August. Another flush of root production has been observed in autumn by Heath and Luckwill (1938). In both studies, root growth is likely to have been underestimated because (i) this information was obtained from field studies using destructive techniques (such as plant harvesting or soil digging) which accounted only for net production of root biomass and did not account for root turnover and (ii) the high specific root length characteristic of ericaceous “hairy” roots implies that fine root growth will lead to substantial

increase in root length while increase in root mass will remain non significant (e.g. see Aerts *et al.*, 1989, for comparison of root biomass and length production in *Erica tetralix*).

Aboveground growth starts in March-April in the British Isles (Ritchie, 1955,1956) but dates of budbreak in other regions depend on latitude and altitude. At budbreak, both species undergo a flush of vegetative and reproductive growth, produced from buds set in the previous growing season. The flowers produced in spring develop into ripe berries within 2 to 3 months, depending on the length of the growing season (Ritchie, 1955,1956; Welch *et al.*, 1994; Lähdesmäki *et al.*, 1990). *V. myrtillus* often exhibits a second period of flowering from axillary buds formed in the current growing season, which can occur in early (Ritchie, 1956) or late summer (Welch *et al.*, 1994) in the British Isles. Secondary flowering events have also been reported in winter for *V. vitis-idaea* (Ritchie, 1955). Although both species produce seeds after either self or insect pollination, seedling germination is rarely observed in their natural environment (Grime *et al.*, 1988).

Considering the highly branched morphology of *V. myrtillus*, the strong apical dominance of *V. vitis-idaea*, and the chamaephytic habit of both species, it seems likely that growth occurs in flushes in order to alternate resource allocation to apical, reproductive, axillary or belowground buds. However, vegetative growth i.e. leaf production, stem elongation and branching has not been studied in any detail. Hence, it is not known if these species display a free or fixed pattern of growth with several flushes, or undergo continuous uninterrupted growth. Leaves of *V. myrtillus* senesce and fall in autumn (between September and November), but some leaves may overwinter on sheltered shoots (Grime *et al.* 1988) probably because no mechanical stress (e.g. wind) induced leaf abscission. It is not known whether leaf senescence in *V. vitis-idaea* displays seasonal trends or is spread all year round.

The apparent lack of determinism in the development of *V. myrtillus* and *V. vitis-idaea* may reflect a high phenotypic plasticity. Ritchie (1956) reported "considerable variation in *V. myrtillus* morphology" in Britain and suggested that it "can be ascribed to phenotypic responses to environmental differences". Tolvanen (1995) found different ramet morphologies when comparing plants growing as forest understorey or in open habitat. In particular, she showed that a greater number of distal buds remained

dormant in plants from the forest habitat as opposed to those in the open habitat. Branching angles appeared to differ between habitats for *V. myrtillus* but not for *V. vitis-idaea*. She also reported that the horizontal growth of the rhizome was enhanced in the open site. She suggested that these morphological differences between the two sites were due to a greater need in the forest site for light interception. She also emphasised the differences between the two species in their branching pattern (sympodial versus monopodial) suggesting different abilities to respond to local stresses such as herbivory (Tolvanen *et al.*, 1992, 1993). These studies highlight the importance of understanding the processes regulating resource allocations at the level of the clone and the ramet in order to explain plant architecture, development and reproduction of *V. myrtillus* and *V. vitis-idaea* in relation with their environment. Since very few data are available on this topic, it might be necessary to restrict the level of study to the ramet, in the first instance, before moving up to the clone level.

III.3 **N nutrition and N internal cycling**

III.3.1 ***Organic N uptake: direct or through mycorrhizal infection***

The extent to which organic N contributes to N uptake of *V. myrtillus* and *V. vitis-idaea* has not been quantified, but uptake of organic nitrogen has been demonstrated in the field for *Vaccinium* species or plants of similar community type (Schimel and Chapin, 1996; Näsholm *et al.* 1998). Kielland (1994) studied the kinetics of uptake of amino acids and ammonium by excised roots of plants from arctic communities in which *V. vitis-idaea* is commonly found. On the basis of these comparative uptake measurements, he suggested that amino acid absorption might account for 10-82 % of the total plant N uptake, depending on the species and its mycorrhizal status. Furthermore, *Vaccinium* species in their natural environment are mycorrhizal, infected by *Hymenoscyphus ericae*. Mycorrhizal infection increases nutrient uptake and thus enhances plant growth via (i) increases in the absorbing surface area, (ii) mobilisation of sparingly available nutrient sources, or (iii) excretion of chelating compounds or ectoenzymes (Marschner and Dell, 1994). In laboratory studies, mycorrhizal infection has been shown to enhance growth and nitrogen uptake of *Vaccinium* species (*Vaccinium macrocarpon* Ait.) by facilitating its access to ammonium-N and organic-N such as amino acids, peptides, chitin and nucleic acids (Read

and Stribley, 1973, Stribley and Read, 1974, 1976, 1980; Bajwa and Read, 1985, 1986; Leake and Read, 1990 and Myers and Leake, 1996). However, the beneficial effect of mycorrhizal infection in terms of N acquisition has costs to the plant in terms of carbon. Between 10 and 20 % of the net gain in plant photosynthates are required for formation, maintenance and function of mycorrhizal structures (Marschner and Dell, 1994). Söderström and Read (1987) reported that up to 30 % of the carbon assimilated by mycorrhizal Pines was used by the symbiotic fungi. Mycorrhizal infection affects not only the N status of the plant, but it alters the pattern of carbon allocation at the whole plant level.

III.3.2 *Mineral root uptake*

Many studies were conducted in the Sixties and early Seventies (e.g. Townsend and Blatt, 1966; Holmes, 1960) on nitrogen assimilation by *Vaccinium* species, with respect to forms of inorganic nitrogen uptake, underlining their preference for ammonium-nitrogen. Some of those authors even consider *Vaccinium* species incapable of assimilating nitrate-nitrogen, assuming an absence of biochemical systems to utilise nitrate. This hypothesis appeared attractive, because it would have been an interesting ecological explanation for the restriction of these species to acid soils, where most of the inorganic nitrogen is available as ammonium-nitrogen (Oertli, 1963). In fact, early attempts to get any evidence of nitrate-reductase (NR) activity in either roots or shoots failed (e.g. Townsend and Blatt, 1966), suggesting an absence of this enzyme or its non-inductibility. Dirr *et al.* (1973) demonstrated that these failures could be due to extraction methodology and measured in vitro activity of NR in leaves of different ericaceous species, including *Vaccinium corymbosum*. Later on, Smirnoff *et al.* (1984) confirmed the ability of *V. myrtillus* and *V. vitis-idaea* to utilise nitrate-N by detecting in vivo NR activity in their leaves after induction by nitrate shoot feeding.

In the field i.e. in their natural environment, *V. myrtillus* and *V. vitis-idaea* are thought to be acquiring N mostly as organic N, due to their mycorrhizal condition. Experimental studies on the nutrition and utilisation of N by these species should include the mycorrhizal component and supply N in organic forms, if they are to be representative of their natural condition. However, the level of mycorrhizal infection would need to be controlled as it affects N status and resource allocation at the plant level. Such a control is difficult to achieve, especially in artificial culture medium (e.g. sand). N

could be supplied in its mineral form only (i.e. nitrate and / or ammonium) since the two species can utilise both ammonium and nitrate. Ingestad (1973) showed that young seedlings of *V. myrtillus* and *V. vitis-idaea* would have a greater growth rate in solution culture when supplied with both sources of mineral N. In addition, he suggested that the two species had the same nutrient requirements and used N at “relatively low concentrations”.

III.3.3 *N storage in Vaccinium species*

The seasonal variations in different N chemical fractions in different plant parts have been studied in both species (Lähdesmäki *et al.*, 1990; Näsholm *et al.*, 1994; Ohlson *et al.*, 1995; Nordin and Näsholm, 1997). The results of these studies suggest that *V. myrtillus* can store N belowground, although no distinction was made between roots and belowground stems. There was no indication in these studies that *V. vitis-idaea* stores N belowground. The possible storage of N by this species in its leaves was not investigated.

IV Aims of this thesis

The aim of the research described in this thesis were to identify the extent to which leaf habit influences the pattern of internal cycling of a species in manner favouring plant adaptation to low N environment. To untangle the effect of leaf habit from that of life form, the two co-generic species, *Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* L., sharing the same growth form but differing in their leaf habit were compared. The patterns of growth and N remobilisation were described at the level of the ramet for each species grown under two levels of N availability (High or Low). Stable isotope techniques were used to distinguish internal from external sources of N (i.e. N remobilised from storage versus N derived from root uptake). The results of three experiments are presented and discussed in chapter 2, 3 and 4, specifically aiming at:

- Establishing differences between species in their growth pattern, dynamics of N remobilisation and sites of N storage when supplied with two different levels of N availability.

- Comparing the effect of internal (i.e. storage) and / or external (i.e. root uptake) availability of N on growth of the two species, and identify the morphological basis of their growth response,
- Determining the extent to which N remobilisation is source or sink driven in each species.

Finally, data are compared across experiments to summarise interspecific differences in the recycling of N within the plant, and in the relationship between N storage and plant development. These differences are discussed in relation to species growth in low N environments.

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