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Nitrogen fixation in perennial forage legumes in the field

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Abstract

Nitrogen acquisition is one of the most important factors for plant production, and N contribution from biological N_2 fixation can reduce the need for industrial N fertilizers. Perennial forages are widespread in temperate and boreal areas, where much of the agriculture is based on livestock production. Due to the symbiosis with N_2-fixing rhizobia, perennial forage legumes have great potential to increase sustainability in such grassland farming systems. The present work is a summary of a large number of studies investigating N_2 fixation in three perennial forage legumes primarily relating to ungrazed northern temperate/boreal areas. Reported rates of N_2 fixation in above-ground plant tissues were in the range of up to 373 kg N ha^{-1} year^{-1} in red clover (*Trifolium pratense* L.), 545 kg N ha^{-1} year^{-1} in white clover (*T. repens* L.) and 350 kg N ha^{-1} year^{-1} in alfalfa (*Medicago sativa* L.). When grown in mixtures with grasses, these species took a large fraction of their nitrogen from N_2 fixation (average around 80%), regardless of management, dry matter yield and location. There was a large variation in N_2 fixation data and part of this variation was ascribed to differences in plant production between years. Studies with experiments at more than one site showed that also geographic location was an important source of variation. On the other hand, when all data were plotted against latitude, there was no simple correlation. Climatic conditions seem therefore to give as high N_2 fixation per ha and year in northern areas (around 60°N) as in areas with a milder climate (around 40°N). Analyzing whole plants or just above-ground plant parts influenced the estimate of N_2 fixation, and most reported values were underestimated since roots were not included. Despite large differences in environmental conditions, such as N fertilization and geographic location, N_2 fixation (Nfix; kg N per ha and year) was significantly ($P < 0.001$) correlated to legume dry matter yield (DM; kg per ha and year). Very rough, but nevertheless valuable estimations of Nfix in legume/grass mixtures (roots not considered) are given by Nfix = 0.026·DM + 7 for *T. pratense*, Nfix = 0.031·DM + 24 for *T. repens*, and Nfix = 0.021·DM + 17 for *M. sativa*.

Abbreviations: ARA – acetylene reduction activity; DM – dry matter; ID – ^{15}N Isotope dilution; NA – ^{15}N Natural abundance; ND – nitrogen difference; Ndfa – N_2 fixation as percent of plant N derived from atmosphere; Nfix – N_2 fixation as kg N per ha and year

Introduction

A good supply of N is always needed for sustainable plant production. Biological N_2 fixation, especially the symbiotic association between legumes and rhizobia, can provide substantial amounts of N to plants and soil, which reduces the need for industrial fertilizers (Ledgard and Steele, 1992; Vance, 1997). In temperate grasslands, perennial forage legumes are very important for the biological supply of N. Due to its tolerance to grazing, white clover (*Trifolium repens* L.) is the most important legume crop in pasture-dominated areas in Europe and New Zealand (Whitehead, 1995). In areas with a long winter period, grasslands are mainly managed as leys for production of winter fod-

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der conserved as hay or silage. In such production, red clover (*T. pratense* L.) and alfalfa (*Medicago sativa* L.) are important legumes (Whitehead, 1995).

Grassland production without input of industrial fertilizers requires optimization of N_2 fixation. The efficiency of N_2 fixing symbioses is a function of host genotype, *Rhizobium* genotype, and environmental factors (Unkovich and Pate, 2000; Whitehead, 1995). Field management practices that ensure favorable conditions for plant growth and bacterial activity are associated with high rates of N_2 fixation (Heichel, 1987; Whitehead, 1995). To evaluate the effects of manipulating field conditions or plant and *Rhizobium* genetics, it is necessary to have proper methods to measure N_2 fixation in field situations (Danso, 1995; Hardarson and Danso, 1993; Peoples et al., 1995). It is also of great importance to have a realistic value of N_2 fixation in different cultivation systems and to understand why activities in field can vary. Unkovich and Pate (2000) provided an updated summary of studies with annual crop legumes. For forage legumes, Ledgard and Steele (1992) reviewed estimations of N_2 fixation and transfer of fixed N from legume to grass in mowed and pastured fields throughout the world. They discussed factors that affect N_2 fixation, with emphasis on pastures. A thorough discussion about N inputs, including reports of N_2 fixation, in relation to sustainability in tropical and temperate cropping systems was given by Peoples et al. (1995). A recent review that focuses on N_2 fixation in forage legume production, in particular *T. pratense*, in temperate/boreal areas seems however to be lacking. This summary provides an overview of field measurements of N_2 fixation in the perennial forage legumes *Trifolium pratense, T. repens* and *Medicago sativa* in the northern temperate/boreal zone, and with a few examples from the southern hemisphere. Some experiments with other crops have been included only as comparison. Published data are analyzed in order to find important factors influencing N_2 fixation, both on an areal basis and at the plant level.

Choice of method to measure N_2 fixation in the field

There are several reviews available which describe and discuss methods to measure N_2 fixation (Danso, 1995; Danso et al., 1993; Hardarson and Danso, 1993; Myrold et al., 1999; Unkovich and Pate, 2000; Weaver and Danso, 1994). Here, we try to point out some important features of the most commonly used methods for field measurements of nitrogen fixation.

^{15}N natural abundance, NA

Because of isotopic discrimination during biological, chemical and physical processes, the natural abundance of the stable isotope ^{15}N in most soils, especially agricultural soils, is slightly higher than in the atmosphere (Bremer and van Kessel, 1990). The deviation from atmospheric ^{15}N abundance is usually presented in δ^{15}N units. One δ^{15}N unit is 1 ‰ deviation from the atmospheric ^{15}N/^{14}N ratio, and the δ^{15}N of atmospheric N_2 is set to 0 (Högberg, 1997; Shearer and Kohl, 1986; Weaver and Danso, 1994). When the δ^{15}N is different in soil and atmosphere, N_2 fixation will alter the ^{15}N/^{14}N ratio in plant tissues compared to plants that rely solely on soil nitrogen, i.e., reference crops. This difference in ^{15}N labeling of plant tissues can be measured with a mass spectrometer and the proportion of legume nitrogen derived from atmosphere (Ndfa) can be calculated. Multiplying Ndfa with the total legume N yield will result in a value of the amount of fixed N_2 over the time period (Hardarson and Danso, 1993; Ledgard and Steele, 1992; Myrold et al., 1999; Unkovich and Pate, 2000; Weaver and Danso, 1994).

A potential problem is that the ^{15}N/^{14}N ratio may be influenced by isotopic discrimination during N_2 fixation and in N transforming processes within the plant (Evans, 2001; Høgh-Jensen and Schjørring, 1994; Ledgard and Steele, 1992; Unkovich and Pate, 2000; Weaver and Danso, 1994; Yoneyama et al., 1986). To take such discrimination into account, the *B* value (the δ^{15}N of the legume grown in absence of combined N), is included when calculating Ndfa (Högberg, 1997). It is proposed that for a reliable quantification the difference between δ^{15}N in the reference crop and *B* should be at least 5 δ^{15}N units (Högberg, 1997; Høgh-Jensen and Schjørring, 1994). The importance of a correct *B* value was demonstrated by Carranca et al. (1999), a negative *B* value resulted in lower estimates than when 0 was used as *B* value in *T. subterraneum*. In *T. repens*, using a *B* value of +1.2 instead of 0 with the NA method increased the values of N_2 fixation with approximately 20% and became similar to the estimates made with ^{15}N isotope dilution (ID, see below) (Høgh-Jensen and Schjørring, 1994; see also Table 9). Isotopic discrimination may be influenced by, e.g., nutrition, moisture and rhizobial genotype (Evans, 2001; Högberg, 1997; Høgh-Jensen and Schjørring, 1994;

Ledgard and Steele, 1992; Riffkin et al., 1999). Unfortunately, few data on *B* are available for perennial forage legumes, especially when older than their first season.

^{15}*N isotope dilution, ID*

With the ID technique the difference between soil and atmosphere in ^{15}N abundance is enlarged through the application of ^{15}N enriched nitrogen fertilizers to the soil. The difference in $^{15}N/^{14}N$ ratios between fixing and non-fixing plants will thus be greater, allowing for more precise estimations of Ndfa than with the NA method.

A difficulty with ID, as well as with NA, is that the labeling of the soil will vary spatially and temporally. This strongly requires that the reference crop and the N_2 fixing crop, in order to take up N with the same $^{15}N/^{14}N$ ratio, follow the same dynamics in N uptake and that they take N from the same soil depth. If not, the variation in soil $^{15}N/^{14}N$ ratio will increase or decrease the difference between fixing and non-fixing plants which leads to errors (Danso et al., 1993; Jacot et al., 2000; Witty, 1983b).

Confounding effects due to transfer of fixed N to neighboring plants?

It has been proposed that if the reference crop is grown in mixture with the fixing crop and benefits from transfer of fixed N, the value of N_2 fixation obtained with the ID or NA method will be underestimated (McNeill and Wood, 1990; Whitehead, 1995). But this error will only occur if there is direct oneway transfer from the legume to the reference crop (Ledgard and Steele, 1992), or if the two crops have different soil N uptake patterns. The major pathways for N transfer, mineralization of dead legume tissues and N excretions via grazing animals, both contribute to a soil N pool that is equally available to the legume and the reference crop. And as long as the legume and the reference crop utilize soil N from the same soil depth and at the same time, additions of fixed N to the soil do not undermine the validity of the NA and ID methods. Moreover, soils in different fields may differ in $^{15}N/^{14}N$ ratio, and if this is the case it will influence the outcome of the NA and ID methods (McNeill and Wood, 1990; Nesheim and Øyen, 1994). It is therefore preferable to always use a non-legume growing in mixture with the legume as reference crop when N_2 fixation in mixed swards is measured.

Total nitrogen difference, ND

With this method, the nitrogen derived from soil is withdrawn from the total amount of plant nitrogen. Estimations of how much N that is derived from soil is achieved by simultaneous cultivation of a non-fixing reference crop, usually grass, in the same field as the N_2 fixing crop. The amount of fixed nitrogen is then assumed to equal the difference between nitrogen accumulated in the fixing crop and the reference crop (Danso, 1995; Ledgard and Steele, 1992). The method is relatively cheap and easy to perform. But the basic assumption with the method, that the two crops absorb soil nitrogen with the same efficiency, may sometimes be severely wrong. In fact, because grasses often use soil N at higher rates than legumes, the use of this method can lead to underestimated, sometimes negative, values of N_2 fixation (Danso, 1995; Hardarson and Danso, 1993; Ledgard and Steele, 1992; Myrold et al., 1999; Unkovich and Pate, 2000; Witty, 1983b).

Acetylene reduction assay, ARA

Acetylene reduction assay is a very sensitive, relatively cheap method, which gives rapid results of analyses and is easy to analyze (Danso, 1995; Vessey, 1994). It is non-destructive, which makes it suitable for following changes in nitrogenase activity in potted plants (Warembourg et al., 1997). The method has however several shortcomings. It is an indirect method; the ratio to convert amounts of acetylene reduced to amounts of N_2 fixed (the C_2H_2/N_2 ratio) depends on a number of factors and it can vary both temporally and between different symbioses. ARA can only measure N_2 fixation over short time periods (minutes or hours). Furthermore the change in N metabolism following incubation in acetylene can cause a decline in nitrogenase activity, leading to underestimation of actual N_2 fixation (Minchin et al., 1983; Witty and Minchin, 1988). Acetylene reduction has been used in field studies (Halliday and Pate, 1976; Mårtensson and Ljunggren, 1984; Masterson and Murphy, 1976; Rice, 1980), but the need for calibration to $^{15}N_2$ incorporation (for the C_2H_2/N_2 ratio) and the short time periods involved makes the method a bad choice for whole-season field measurements. For such measurements, ^{15}N techniques are now largely applied in favour of ARA (Ledgard and Steele, 1992).

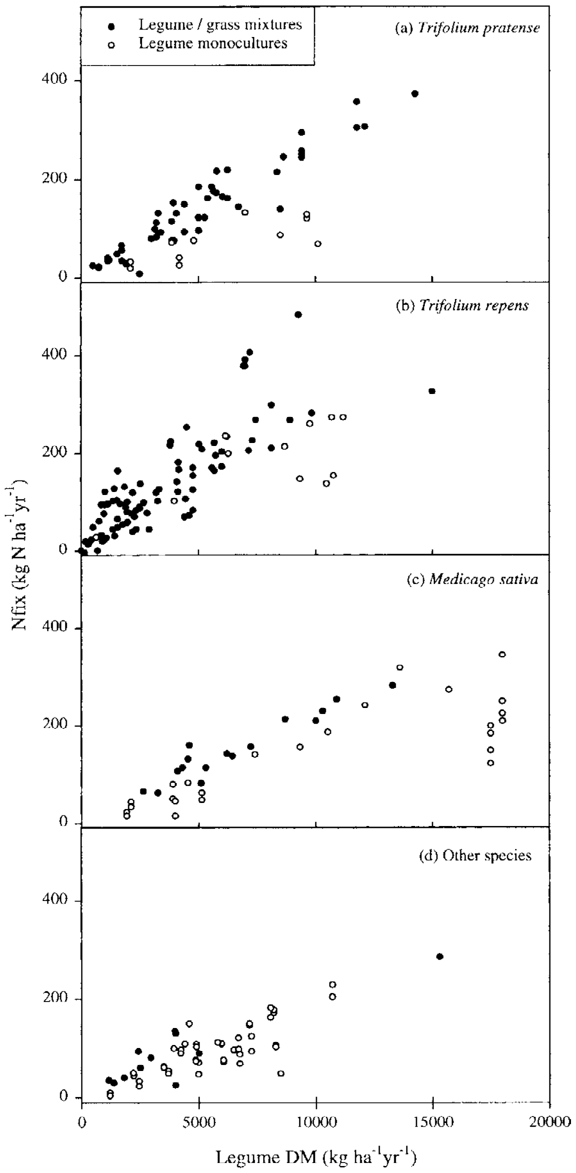

Figure 1. N_2 fixation related to legume dry matter yield. Data included are collected from the studies presented in Tables 1, 2, 4 and 5. Each point represents results from one treatment or year in a specific study.

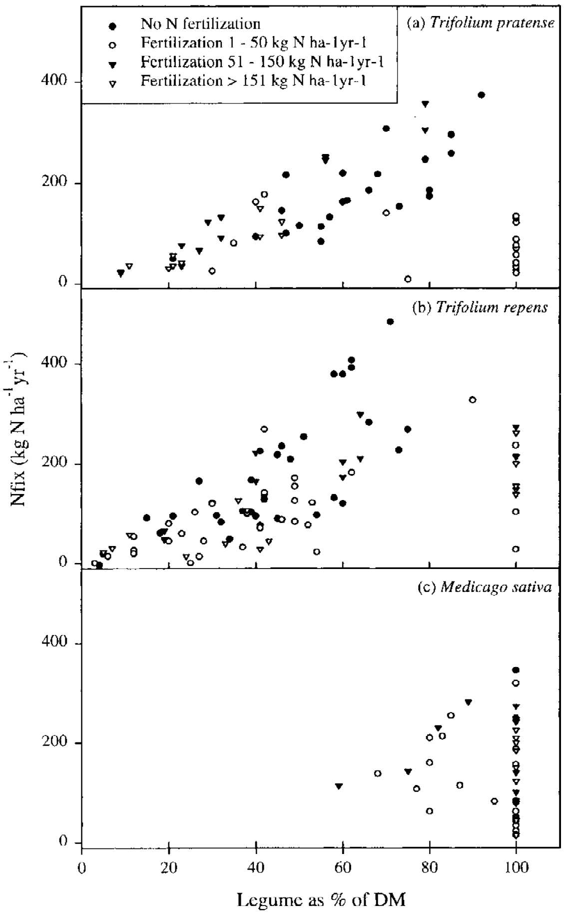

Figure 2. N_2 fixation related to legume DM yield as percent of total DM yield. Further details, see legend of Figure 1.

Important aspects regarding perennial forage legumes

Perennial forage legumes carry stored N in their roots. The amount and $^{15}N/^{14}N$ ratio of this stored N reflect N_2 fixing activity and discrimination during N transformations in preceding years. Estimations of N_2 fixation made with the NA method might therefore be influenced by N assimilated in the past. With ID, the addition of ^{15}N to the system represents the starting point of the experiment. Stored N does therefore not affect results obtained with ID, provided that the perennial reference crop and the fixing crop have similar proportions of their N coming from storage. The $^{15}N/^{14}N$ ratio in roots may differ from that in shoots because of isotopic discrimination (Carranca et al., 1999; Danso et al., 1993; Evans, 2001; Shearer and Kohl, 1986; Yoneyama et al., 1986). It is therefore also important that the reference crop and the fixing crop have equal distributions of ^{15}N between root and shoot, unless whole plants are used. In conclusion, as long as appropriate reference crops are used, NA and ID are the most suitable methods for whole-season field measurements of N_2 fixation.

Overview of data

The range of N_2 fixation values (kg N ha^{-1} year^{-1} in

Table 1. Summary of N_2 fixation (kg N ha^{-1} $year^{-1}$) in *Trifolium pratense*. Data are listed according to decreasing latitude. Swards were kept under a cutting management, except when noted 'Grazing'

Range	Method[a]	Cause of variation	Stand description and reference crops	Geographic location, latitude	Reference
35–185	ID & ND	2 years, method, 0 or 160 kg N[b]	Mixture with *Phleum pratense*[c]	Norway, 67°N	Nesheim and Øyen, 1994
14–99	ND	4 years, ley age, organic or conventional farming	Mixture with *Phleum pratense*, *Festuca pratensis* and *Trifolium repens*	Sweden, 65°N	Fagerberg and Sundqvist, 1994
16–149[d]	ID & ND	2 years, reference crop, method, site	Monoculture, *T. pratense*[ce], *Medicago sativa*[ce], *Brassica oleracea*[c], *Hordeum vulgare*[c]	Alaska, 64°N	Sparrow et al., 1995
30–100	ND	Many years, ley age, site, 0 or 300 kg N[b]	Mixture with *Phleum pratense, Festuca pratensis*	Sweden, 63–66°N	Gustavsson, 1989
94–295	ID & ND	2 years, method, 0 or 160 kg N[b]	Mixture with *Phleum pratense*[c]	Norway, 59°N	Nesheim and Øyen, 1994
217	ID		Mixture with *Lolium perenne*[c]. Grazing	Denmark, 56°N	Vinther and Jensen, 2000
21–61	ID & ND	Reference crop, method	Monoculture, *Lolium perenne*[c], *Brassica rapa*[c]	England, 51°N	Witty, 1983a
76–215	ID	2 clover strains, 0 or 150 kg N[b]	Mixture with *Lolium multiflorum*[c]	Switzerland, 47°N	Boller and Nösberger, 1994
21–373	ID & ND	2 sowing years, 2 production years, method, 0 or 120 kg N[b]	Mixture with *Lolium multiflorum*[c]	Switzerland, 47°N	Boller and Nösberger, 1987
8–150[d]	ID	4 years	Mixture with *Phalaris arundinacea*[c]	Minnesota, 45°N	Heichel and Henjum, 1991
69–133[d]	ID	4 years	Monoculture, *Phalaris arundinacea*[c]	Minnesota, 45°N	Heichel et al., 1985
73–159	ID	2 years, 4 cultivars	Mixture with *Dactylis glomerata*[c]	Iowa, 42°N	Farnham and George, 1993
66–258	ID	2 years, 4 cultivars, 3 or 6 cuts $year^{-1}$	Mixture with *Dactylis glomerata*[c]	Iowa, 42°N	Farnham and George, 1994
162–177	ID	1 sowing year, 1 production year	Mixture with *Lolium perenne*[c]. Grazing	New Zealand, 37°S	Ledgard et al., 1990

[a]ID – ^{15}N isotope dilution; NA – ^{15}N natural abundance; ND – nitrogen difference.
[b]kg N fertilization ha^{-1} $year^{-1}$.
[c]Indicates reference crop.
[d] Above- and below-ground harvests included.
[e]Noninoculated, nonnodulating or ineffective plant variety.

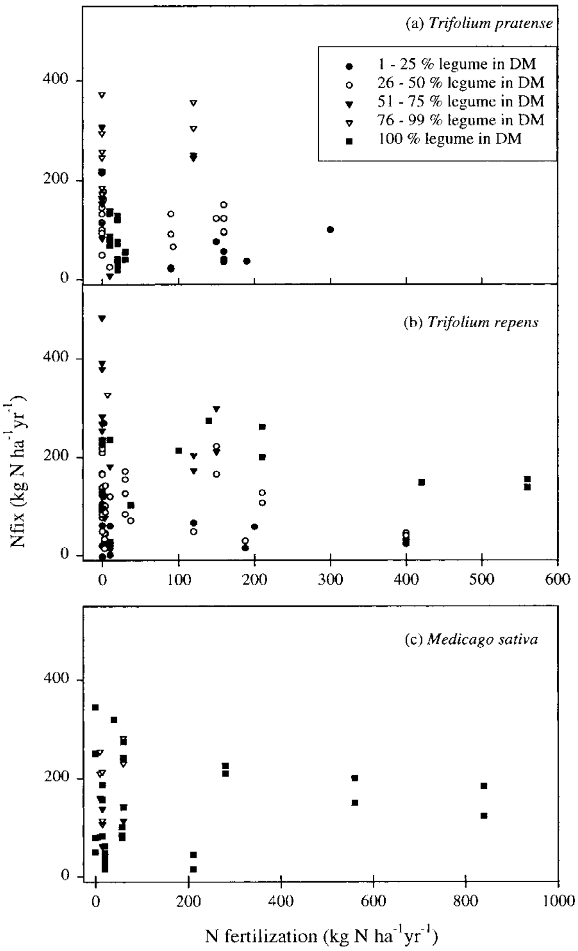

Figure 3. N_2 fixation related to N fertilization. Note different scales on *x*-axes. Further details, see legend of Figure 1.

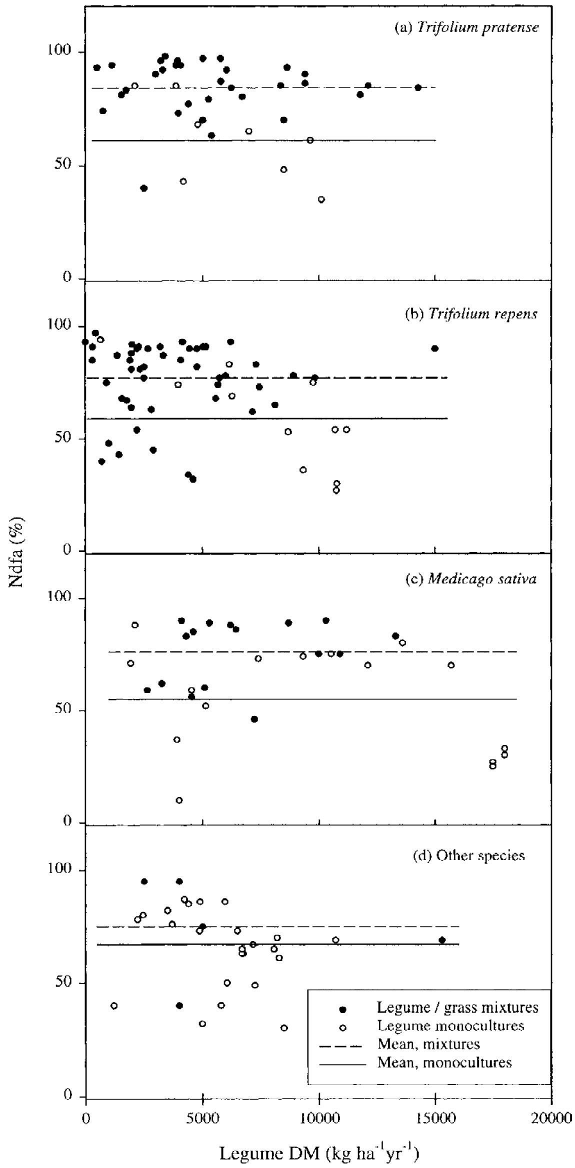

Figure 4. Ndfa related to legume dry matter yield. Further details, see legend of Figure 1.

shoots) is summarized for *Trifolium pratense* (Tables 1 and 3), *T. repens* (Tables 2 and 3), *Medicago sativa* (Table 4) and other legume species (Table 5). The magnitude of N_2 fixation can be up to 373 kg N ha^{-1} year^{-1} in *T. pratense*, 545 kg N ha^{-1} year^{-1} in *T. repens* and 350 kg N ha^{-1} year^{-1} in *Medicago sativa*. In this summary, only estimates of N_2 fixation obtained with NA, ID or ND are included. The variation is large both among studies and within studies. In the following we analyze the importance of legume biomass, N fertilization, year, plant genotype, methods, and site on reported N_2 fixation values. Statistical tests for difference (*T*-test) and correlation are made according to Minitab 13.0 statistical software, Minitab Inc.

Importance of legume biomass and N fertilization on N_2 fixation

Data in the studies presented in Tables 1, 2, 4 and 5 are used to investigate the relationships between N_2 fixation and legume dry matter yield (Figures 1 and 4), legume proportion of stand (Figures 2 and 5), and N fertilization (Figures 3 and 6). N_2 fixation is expressed as kg N ha^{-1} year^{-1}, hereafter referred to as Nfix (Figures 1–3) or as Ndfa (Figures 4–6). Furthermore,

Table 2. Summary of N_2 fixation (kg N ha^{-1} $year^{-1}$) in *Trifolium repens*. Data are listed according to decreasing latitude. Swards were kept under a cutting management, except when noted 'Grazing'

Range	Method[a]	Cause of variation	Stand description and reference crops	Geographic location, latitude	Reference
60–120	ID	2 years	Mixture with *Lolium perenne*[c]. Grazing	Denmark, 56°N	Hansen and Vinther, 2001
15–235	ID	2 sowing years, 3 production years, ley age, 0–225 kg N[b], 1–7 cuts $year^{-1}$	Mixture with *Lolium perenne*[c]. Grazing	Denmark, 56°N	Vinther and Jensen, 2000
50–120	ID	2 years, 0 or 40 Mg urine ha^{-1} $year^{-1}$	Mixture with *Lolium perenne*[c]	Denmark, 56°N	Vinther, 1998
47–140	ID & NA	3 years, 3–72 kg N[b], stand composition	Monoculture or mixture with *Lolium perenne*[cf]	Denmark, 55°N	Høgh-Jensen and Schjørring, 1997
23–262	ID	1 sowing year, 2 production years, stand composition	Monoculture or mixture with *Lolium perenne*[cf]	Denmark, 55°N	Jørgensen et al., 1999
27–122[d]	ID	3 sites (lowland–upland)	Mixture with *Lolium perenne*[c]	Wales, 52°N	Goodman, 1988
81–100	ND	Cutting or grazing, 2 sites	Mixture with *Lolium perenne*. Cutting/grazing	Wales, 52°N	Munro and Davies, 1974
150–545	ND	4 years, 3 clover cultivars, 2 grass cultivars	Mixture with *Lolium perenne*	The Netherlands, 52°N	Elgersma and Hassink, 1997
217–445	ND	4 years, 3 clover cultivars, 2 grass cultivars	Mixture with *Lolium perenne*	The Netherlands, 52°N	Elgersma et al., 1998
84–171	ID & ND	Method, reference crop in monoculture or mixed with clover	Mixture with *Lolium perenne*[c]	England, 51°N	McNeill and Wood, 1990
70–351	ID	3 years, 100—560 kg N, stand composition, ambient or elevated pCO_2	Monoculture or mixture with *Lolium perenne*[c]	Switzerland, 47°N	Zanetti et al., 1996
48–299	ID & ND	2 sowing years, 2 production years, method, 0 or 150 kg N[b]	Mixture with *Lolium perenne*[c]	Switzerland, 47°N	Boller and Nösberger, 1987
102–282	ID	2 years, cutting height, stand composition	Monoculture or mixture with *Lolium perenne*[cf]	Switzerland, 47°N	Seresinhe et al., 1994b
1–20[d]	ID	4 years	Mixture with *Phalaris arundinacea*[c]	Minnesota, 45°N	Heichel and Henjum, 1991
327[d]	ID		Monoculture, weeds[c]	New Zealand, 43°S	Kumar and Goh, 2000
45–142	ID	Age of pasture	Mixture with grasses[c] (mostly *Lolium perenne*). Grazing	New Zealand, 43°S	Edmeades and Goh, 1978
54–80	ID	2 sites	Mixture with *Lolium perenne* and *Agrostis cappilaris*. Grazing	New Zealand, 37°S	Ledgard et al., 1987
82–291	ID	1 sowing year, 1 production year, 4 clover cultivars	Mixture with *Lolium perenne*[c]. Grazing	New Zealand, 37°S	Ledgard et al., 1990
10–120	ID	5 years, 0–400 kg N[b], grazing intensity	Mixture with *L. perenne*[c] (and weeds). Grazing	New Zealand, 37°S	Ledgard et al., 2001

[a]ID – ^{15}N isotope dilution; NA – ^{15}N natural abundance; ND – nitrogen difference.
[b]kg N fertilization ha^{-1} $year^{-1}$.
[c]Indicates reference crop.
[d]Above- and below-ground harvests included.
[f]Indicates that reference crop is in monoculture.

Table 3. Summary of N_2 fixation (kg N ha^{-1} $year^{-1}$) in *Trifolium pratense* and *Trifolium repens* in mixture, and in various perennial *Trifolim* species (*Trifolium* spp.). In all studies, swards were kept under a cutting management

Range	Method[a]	Cause of variation	Stand description and reference crops	Geographic location, latitude	Reference
Trifolium pratense & *T. repens* mixture					
46–171	ID & ND	Cutting frequency, method, 0 or 400 kg N[b]	Mixture with *Lolium perenne*[cf]	Denmark, 55°N	Høgh-Jensen and Kristensen, 1995
31–161	ID & NA	Cutting frequency, method, 20 or 400 kg N[b]	Mixture with *Lolium perenne*[cf]	Denmark, 55°N	Høgh-Jensen and Schjørring, 1994
Trifolium spp.					
0–293	ND	Many years, different fields, organic or conventional farming	Mixture with grasses in leys	Sweden, 59°N	Granstedt, 1990
0–314	ND	Many years, different fields, organic or conventional farming	Mixture with grasses in leys	Sweden, 55°N	Granstedt, 1990

[a]ID – ^{15}N isotope dilution; NA – ^{15}N natural abundance; ND – nitrogen difference.
[b]kg N fertilization ha^{-1} $year^{-1}$.
[c]indicates reference crop.
[f]Indicates that reference crop is in monoculture.

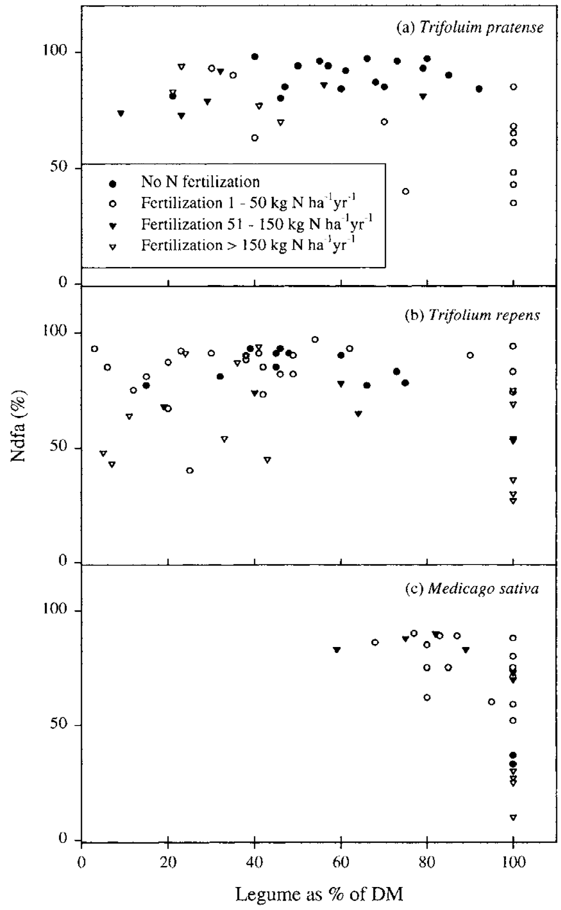

Figure 5. Ndfa related to legume DM yield as percent of total DM yield. Further details, see legend of Figure 1.

the influence of N fertilization on legume DM yield is examined (Figure 7).

N_2 fixation per ha and year

There is a positive correlation between Nfix and legume dry matter yield in *Trifolium* spp. and *M. sativa* ($P < 0.001$, Figure 1a–c). The correlation is true also for 'other species' ($P < 0.001$, Figure 1d), which includes annual grain legumes as well as annual and perennial forage legumes from very different locations and management regimes. Obviously, plant productivity is a very important factor determining the amount of N_2 fixed. In most cases, at a given DM yield, legume/grass mixtures reach higher Nfix values than legume monocultures (Figure 1a–c). For *T. pratense* and *T. repens*, Nfix is also positively correlated to legume DM yield as percent of DM yield in grass-legume mixtures (Figure 2a, b).

Studies designed to compare different fertilization regimes showed that increased N fertilization always suppressed N_2 fixation (Boller and Nösberger, 1987, 1994; Gustavsson, 1989; Høgh-Jensen and Schjørring, 1994; Nesheim and Øyen, 1994; Vinther and Jensen, 2000). But there is no simple correlation between N_2 fixation (per ha and year) and N fertilization when the complete data set is used (Figure 3). Such N fertilization effects are probably hidden among other sources of variation (genotypes, sites, management, etc.) when using the entire data set (Figure 3).

Models to calculate Nfix based on DM yield

Equations derived from the correlations in Figure 1 can be used for estimations of Nfix based on legume DM yield (Table 6). Due to a large variation in the data used, these equations give only a rough estimation of N_2 fixation. Nevertheless, this simple way of calculating Nfix can be of great value from a practical point of view, e.g., for estimating the amount of N provided by a legume in a farmer's field. The relationships between Nfix and DM yield are stronger for legume/grass mixtures than for legume monocultures (Table 6, R^2-values), so it may be more accurate to apply these models to mixtures than to monocultures. Kristensen et al. (1995) proposed a simple model for estimations of Nfix in clover/grass mixtures based on clover proportion and age of the sward. In their model, they used data from three investigations performed with the ND method in Denmark. The models shown in Table 6 can be applied in a wider range of situations than the model of Kristensen et al. (1995), since they are derived from many different locations and management regimes and include NA, ID and ND methods.

Ndfa

N_2 fixation expressed as Ndfa often reaches high values over a large range of legume dry matter yields, single values approach 100% (Figure 4). *Trifolium pratense*, *T. repens* and *M. sativa* all achieve a higher proportion of their N from N_2 fixation when grown in mixture with grasses than when they are grown in monoculture ($P < 0.05$, Figures 4 and 5). In legume/grass mixtures, grasses have been found to be stronger competitors for soil N, and legumes rely more on N_2 fixation as N source (Loiseau et al. 2001; Zanetti et al. 1996).

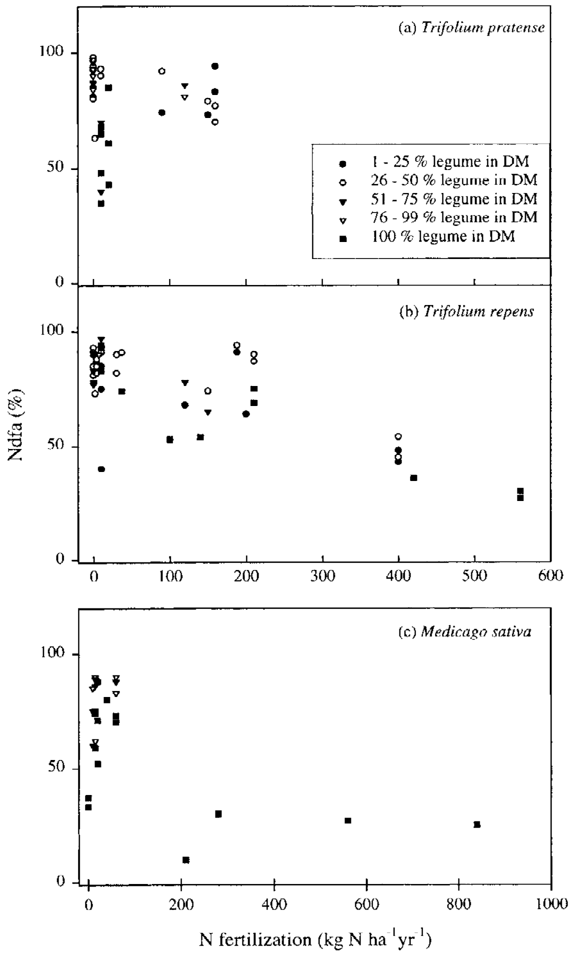

Figure 6. Ndfa related to N fertilization. Note different scales on *x*-axes. Further details, see legend of Figure 1.

Trifolium repens and *M. sativa* monocultures have lower Ndfa values at high applications of N fertilizer, while in *T. pratense*, no Ndfa data are available at N fertilization rates above 200 kg N ha^{-1} year^{-1} (Figure 6). When no N fertilizer is applied, *T. pratense* often constitute a large proportion of DM yield in mixtures with grasses and have high Ndfa values (Figure 5a). On the other hand, high N fertilization (>150 kg N ha^{-1} year^{-1}) restricts clover proportions in clover/grass mixtures to ca. 50% or less, for both *T. pratense* and *T. repens* (Figures 2a, b, 3a, b and 5a, b). When these species are grown in mixtures with grasses, N fertilization apparently gives the grass component an advantage while the clover proportion of the yield is decreased (Boller and Nösberger, 1987; Gustavsson, 1989; Høgh-Jensen and Kristensen, 1995; Jørgensen and Ledgard, 1997;

Table 4. Summary of N_2 fixation (kg N ha^{-1} year^{-1}) in *Medicago sativa*. Data are listed according to decreasing latitude. Swards were kept under a cutting management, except when noted 'Grazing'

Range	Method[a]	Cause of variation	Stand description and reference crops	Geographic location, latitude	Reference
(-5)–72[d]	ID	2 years, reference crop, method, site	Monoculture, *Medicago. sativa*[ce], *Brassica oleracea*[c], *Hordeum vulgare*[c]	Alaska, 64°N	Sparrow et al., 1995
79–101	ID, ND,	Method, reference crop	Monoculture, *Festuca pratensis*[c], *M. sativa*[ce]	Sweden, 60°N	Mårtensson and Ljunggren, 1984
242–319	ID	2 years	Monoculture, *Festuca pratensis*[c]	Sweden, 60°N	Wivstad et al., 1987
65–213	ID	3 years, stand composition	Monoculture or mixture with *Bromus riparius*[cf]	Canada, 51°N	Walley et al., 1996
197–222	ID	2 years, 5–45 kg N[b], reference crop	Mixture with *Lolium perenne*[c]	Austria, 48°N	Danso et al., 1988
114–282	ID	2 years, plant density	Monoculture or mixture with *Lolium perenne*[cf]	Austria, 48°N	Hardarson et al., 1988
93–258	ID	3 years	Monoculture or mixture with *Phleum pratense*[c] and *Bromus inermis*[c]	Canada, 45°N	Burity et al., 1989
82–254[d]	ID	4 years	Mixture with *Phalaris arundinacea*[c]	Minnesota, 45°N	Heichel and Henjum, 1991
123–350	ID, ND	Method, 0–840 kg N[b]	Monoculture, *M. sativa*[ce]	Minnesota, 45°N	Lamb et al., 1995
10–140	NA	3 years, plant density	Mixture with *Trifolium subterraneum*[c] and *Phalaris arundinacea*[c]. Grazing	Australia, 35°S	Dear et al., 1999

[a]ID – ^{15}N isotope dilution; NA – ^{15}N natural abundance; ND – nitrogen difference.
[b]kg N fertilization ha^{-1} year^{-1}.
[c]indicates reference crop.
[d]Above and below-ground harvests included.
[e]Noninoculated, nonnodulating or ineffective plant variety.
[f]Indicates that reference crop is in monoculture.

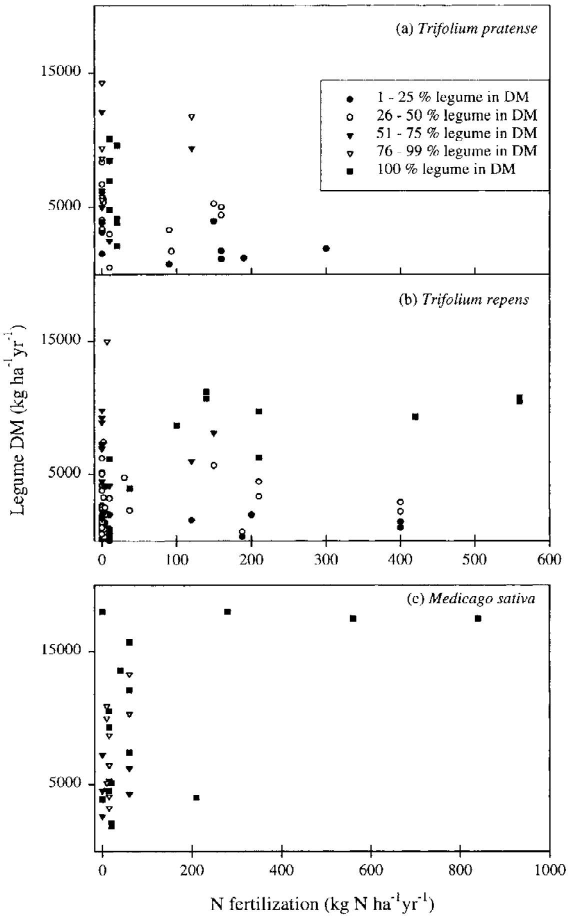

Figure 7. Legume dry matter yield related to N fertilization. Note different scales on *x*-axes. Further details, see legend of Figure 1.

Ledgard et al., 2001; Loiseau et al., 2001; Nesheim and Øyen, 1994). It is important to point out that a decrease in clover proportion is not synonomous to a lower clover DM yield, i.e., N fertilization may increase grass growth and total DM yield of the mixture while clover DM yield remains unchanged, thus decreasing clover proportion.

The effect of N fertilization on DM yield in *T. pratense* and *T. repens* is very similar to the effect on Nfix, showing again the close relationship between dry matter yield and N_2 fixation per ha and year in these species (Figures 1a, b and 7a, b). *Medicago sativa* in monoculture maintains a high DM yield while Ndfa is decreased at high N fertilization (Figures 6c and 7c, Lüscher et al., 2000).

Sources of variation in N_2 fixation

Variation between years

Environmental conditions such as temperature and rainfall can differ considerably from year to year, causing large variations in plant production and N_2 fixation. In Table 7, studies with multi-year experiments are summarized. Some experiments were repeated over years without resowing, thus causing variation both due to increasing age of stand and due to different conditions in different years. Variation associated with such repetitions are seen in studies that have data in several columns in Table 7 (e.g., Heichel and Henjum, 1991; Nesheim and Øyen, 1994). Other experiments were repeated by resowing in another year, hence removing the effect of age of stand. This kind of variation is illustrated by dividing a study into several rows in Table 7 (e.g., Sparrow et al., 1995). In some studies both ways of repetition were used, and they show the pure effect of age of stand, since fields of different age can be compared the same year (Table 7, Jørgensen et al., 1999; Vinther and Jensen, 2000). It is obvious that variation in environmental conditions (weather, etc.) between years, as well as varying age of stands cause great differences in Nfix and DM production, often more than 2-fold (Table 7). Nfix and legume DM yield are usually lower in the sowing year than in production years (Table 7, e.g., Boller and Nösberger, 1987; Jørgensen et al., 1999; Vinther and Jensen, 2000; Walley et al., 1996). Variations in Nfix and in DM yield are large, both for age of stand and for different sowing years, while the differences between years in Ndfa are considerably smaller (Table 7). This implies that environmental conditions affect N_2 fixation mainly through effects on plant production, and that Ndfa is less affected.

Variation between species and varieties

Several studies have compared different legume species or varieties with respect to N_2 fixation (Table 8). On average, Nfix in *M. sativa* is 45 (27) kg larger than in *T. pratense*, and 167 (34) kg larger than in *T. repens* (standard error of means in brackets). Nfix in *T. pratense* is on average 23 (24) kg larger than in *T. repens*. Differences in Nfix are large between species but also between varieties, obviously the plant genotype can have large effects on N_2 fixation. Moreover, variations in Nfix and DM yield are larger than variations in Ndfa (Table 8). This implies that host genotypic

Table 5. Summary of N_2 fixation (kg N ha^{-1} $year^{-1}$) in other legume species. Data are listed according to decreasing latitude. Swards were kept under a cutting management, except when noted 'Grazing'

Species	Range	Method[a]	Cause of variation	Stand description and reference crops	Geographic location, latitude	Reference
Melilotus officinalis	4–123[d]	ID, ND	2 years, reference crop, method, site	Monoculture, *Medicago officinalis*[ce], *M. sativa*[ce], *Brassica oleracea*[c], *Hordeum vulgare*[c]	Alaska, 64°N	Sparrow et al., 1995
Vicia faba	82–249[d]	ID, ND	2 years, reference crop, method, site	Monoculture, *Vicia faba*[ce], *M. sativa*[ce], *Brassica oleracea*[c], *Hordeum vulgare*[c]	Alaska, 64°N	Sparrow et al., 1995
Pisum sativum	42–144[d]	ID, ND	2 years, reference crop, method, site	Monoculture, *Pisum. sativum*[ce], *M. sativa*[ce], *Brassica oleracea*[c], *Hordeum vulgare*[e]	Alaska, 64°N	Sparrow et al., 1995
Lens culinaris	21–91[d]	ID, ND	2 years, reference crop, method, site	Monoculture, *Lens culinaris*[ce], *M. sativa*[ce], *Brassica oleracea*[c], *Hordeum vulgare*[c]	Alaska, 64°N	Sparrow et al., 1995
Lupinus alba	33–202[d]	ID, ND	2 years, reference crop, method, site	Monoculture, *Lupinus alba*[ce], *M. sativa*[ce], *Brassica oleracea*[c], *Hordeum vulgare*[c]	Alaska, 64°N	Sparrow et al., 1995
Vicia faba	160–238	ID, ND	Reference crop, method	Monoculture, *Lolium perenne*[c], *Brassica rapa*[c]	England, 51°N	Witty, 1983a
Pisum sativum	53–97	ID, ND	Reference crop, method	Monoculture, *Lolium perenne*[c], *Brassica rapa*[c]	England, 51°N	Witty, 1983a
Phaseolus vulgaris	84–131	ID, ND	Reference crop, method	Monoculture, *Lolium perenne*[c], *Brassica rapa*[c]	England, 51°N	Witty, 1983a
L. corniculatis	25–130[d]	ID	4 years	Mixture with *Phalaris arundinacea*[c]	Minnesota, 45°N	Heichel and Henjum, 1991
L. corniculatis	49–112[d]	ID	4 years	Monoculture, *Phalaris arundinacea*[c]	Minnesota, 45°N	Heichel et al., 1985
T. subterraneum	50–150	NA	3 years	Monoculture, *Phalaris arundinacea*[c]. Grazing	Australia, 35°S	Dear et al., 1999
T. subterraneum	30–170	NA	3 years, plant density	Mixture with *M. sativa*, *P. arundinacea*[cf]. Grazing	Australia, 35°S	Dear et al., 1999
T. subterraneum	20–100	NA	3 years, plant density	Mixture with *Phalaris arundinacea*[cf]. Grazing	Australia, 35°S	Dear et al., 1999
Pisum sativum	286[d]	ID		Monoculture, weeds[c]	New Zealand, 43°S	Kumar and Goh, 2000

[a]ID – ^{15}N isotope dilution; NA – ^{15}N natural abundance; ND – nitrogen difference.
[c]Indicates reference crop.
[d]Above and below-ground harvests included.
[e]Noninoculated, nonnodulating or ineffective plant variety.
[f] Indicates that reference crop is in monoculture.

Table 6. Equations for calculation of Nfix, generated from data in Figure 1. R^2, correlation coefficient

	All observations		Legume/grass mixtures		Legume monocultures	
Species	Nfix =	R^2	Nfix =	R^2	Nfix =	R^2
T. pratense	0.023 * DM + 8.4	0.71	0.026 * DM + 7.4	0.91	0.010 * DM + 16.5	0.55
T. repens	0.025 * DM + 37.2	0.63	0.031 * DM + 23.9	0.71	0.016 * DM + 57.9	0.47
M. sativa	0.012 * DM + 38.8	0.62	0.021 * DM + 16.9	0.91	0.013 * DM + 12.3	0.70
Other spp.	0.017 * DM + 7.3	0.68	0.017 * DM + 21.1	0.83	0.017 * DM − 0.65	0.64

effects on N_2 fixation are mainly governed by plant growth.

Variation due to method used to measure N_2 fixation

Different methods have been used in parallel in some studies (Table 9). The two methods most frequently compared are ID and ND, and there are often considerable differences between the estimates obtained. Only nine out of 22 experiments show good correspondence between the two methods (ND/ID = 0.9–1.1). The poorest correspondence is an ND/ID value of 0.54 (Table 9). Studies where ND gives higher values or lower values than ID are equally common (Table 9). With the ND method there is a risk for underestimation of N_2 fixation, since grasses usually are more efficient in taking up soil N than legumes (Hardarson and Danso, 1993; Ledgard and Steele, 1992; Myrold et al., 1999; Unkovich and Pate, 2000; Witty, 1983b). Compared to ID, ND often gave lower estimates of Nfix when N yield in grass monocultures were compared with N yield in legume monocultures (Table 9). Legume–grass mixtures on the other hand may utilize soil N more efficiently, and thus reach higher total N yields (Boller and Nösberger, 1987; Høgh-Jensen and Schjørring, 1994, 1997). It is also possible that species composition influences soil microbial processes, such as mineralization, with indirect effects on soil N availability (Spehn et al., 2000). Subtracting the N yield in a grass monoculture from the total N yield in a legume/grass mixture can therefore lead to an overestimation of Nfix. In Table 9, ND/ID values from experiments where grass monocultures were compared with legume/grass mixtures are significantly higher ($P < 0.001$) than the ND/ID values obtained in studies where grass monocultures were compared with legume monocultures. The outcome of the ND method thus seems to be dependent on the stand composition: for monocultures ND can lead to underestimations, and for mixtures ND can lead to overestimations of N_2 fixation, as compared to ID.

Variation due to plant parts analyzed

In most studies the estimate of N_2 fixation is based only on aboveground plant parts. Excluding roots from the measurement will not give an estimate of total N_2 fixation, but the amount of the fixed N that is allocated to harvested plant parts (i.e., above-ground). Depending on plant species, different proportions of the fixed N are located in the roots. According to Danso et al. (1993), grain legumes have less than 10% of their DM, and therefore a low proportion of total plant fixed N, in the root system. Forage legumes, in contrast, may have up to 60% of their fixed N in the root system (Danso et al., 1993). Plant root/shoot ratio is also influenced by, e.g., soil water status and nutrients, especially P. Both drought stress and P deficiency have been reported to increase root/shoot ratio (Marschner, 1995). In a glass-house study, Jørgensen and Ledgard (1997) found that N fertilization reduced the root/shoot ratio in *T. repens*. Furthermore, they conclude that about 60% of total fixed N in *T. repens* is allocated to above cutting height tissues. Amounts of fixed N in plant parts above and below cutting height have been estimated for *T. repens* and *M. sativa* (Table 10). The proportion of total fixed N in the plant that is located above cutting height varies considerably, from 36 to about 90% (Table 10). There are however differences in how these pools are calculated. Amounts of fixed N in shoots are mostly calculated from the seasonal shoot yields while the corresponding values for roots are often calculated from existing root biomass in perennial stands (Table 10). In other words, annual or seasonal shoot N pools are compared with root N pools resulting from build-up and turnover during several years. While annual shoot growth is easily recorded as harvests, annual root growth in a multi-year stand is much more difficult to measure and it is therefore

Table 7. Variation between years in N_2 fixation (Nfix, kg N ha^{-1} $year^{-1}$), Ndfa (%) and legume dry matter yield (DM, ton ha^{-1} $year^{-1}$). < 20 kg N fertilization ha^{-1} $year^{-1}$, ID, except when noted differently. Sowing year is the year when the field is established. Production years are the years following the sowing year, providing that the field is not resown

Species		Sowing year[a]			Production year 1			Production year 2			Production year 3			Production year 4		Reference
	Sowing year	Nfix	Ndfa	DM	Nfix	Ndfa	DM	Nfix	Ndfa	DM	Nfix	Ndfa	DM	Nfix	DM	
T. pratense	1988				173	97	5.8	83	96	3.2						Nesheim and Øyen, 1994, Vågønes
T. pratense	1988				258	90	9.4	162	84	6.3						Nesheim and Øyen, 1994, Særheim
T. pratense	1988	121	61	9.6												Sparrow et al., 1995, Fairbanks
T. pratense	1989	41	43	4.2												Sparrow et al., 1995, Fairbanks
T. pratense	1988	76	85	3.8												Sparrow et al., 1995, Delta Junction
T. pratense	1989	33	85	2.1												Sparrow et al., 1995, Delta Junction
T. pratense	1983	165	92	6.0	373	84	14.3									Boller and Nösberger, 1987
T. pratense	1984	49	81	1.5	307	85	12.1									Boller and Nösberger, 1987
T. pratense	1982	8	40	2.5	150	70	8.5	80	90	3.0	25	93	0.5			Heichel and Henjum, 1991
T. pratense	1977	133	65	7.0	69	35	10.1	87	48	8.5	76	68	4.8			Heichel et al., 1985
T. pratense	1988				153	96	3.9	93	98	3.4						Farnham and George, 1993
T. pratense	1989				216	95	6.8	124	94	4.5						Farnham and George, 1994
T. pratense	1986	177		5.4	162	63	5.4									Ledgard et al., 1990
T. repens	1993				60	92	2.0	120	91	3.2						Hansen and Vinther 2001[b]
T. repens	1994	29	94	0.6	235	93	6.2	100	90	2.7						Vinther and Jensen, 2000
T. repens	1995	15	91	0.3	167	93	4.2									Vinther and Jensen, 2000
T. repens	1993				115	83	4.0	140	78	5.1	72	78	2.6			Høgh-Jensen and Schjørring, 1997, monoculture
T. repens	1993				65	95	2.0	114	94	3.4	71	93	2.2			Høgh-Jensen and Schjørring, 1997, mixture
T. repens	1994	38	94	1.0	274	80	6.7	192	78	5.6						Jørgensen et al., 1999, monoculture
T. repens	1995	17	94	0.3	249	88	6.4	230	85	6.0						Jørgensen et al., 1999, monoculture
T. repens	1994	30	98	0.6	188	94	4.4	158	88	4.0						Jørgensen et al., 1999, mixture
T. repens	1995	15	96	0.3	286	96	4.3	195	92	4.0						Jørgensen et al., 1999, mixture
T. repens	1991				483		9.3	392		7.0	225		3.8	379	6.9	Elgersma and Hassink, 1997, ND
T. repens	1991				393		7.1	236		4.2						Elgersma et al., 1998[c], ND
T. repens	1983	227	83	7.3	268	78	8.9									Boller and Nösberger, 1987
T. repens	1984	83	81	2.3	283	77	9.8									Boller and Nösberger, 1987
T. repens	1989				260	75	9.8	200	69	6.3						Seresinhe et al., 1994[d], monoculture
T. repens	1989				106	90	4.5	127	87	3.3						Seresinhe et al., 1994[d], mixture
T. repens	1982	1	40	0.7	20	75	0.9	15	85	0.3	1	93				Heichel and Henjum, 1991
T. repens	1986	103		3.3	269	73	7.4									Ledgard et al., 1990

Table 7. Continued.

Species	Sowing year	Sowing year[a]			Production year 1			Production year 2			Production year 3			Production year 4		Reference
		Nfix	Ndfa	DM	Nfix	Ndfa	DM	Nfix	Ndfa	DM	Nfix	Ndfa	DM	Nfix	DM	
M. sativa	1988	58	49	5.0												Sparrow et al., 1995, Fairbanks
M. sativa	1989	63	56	5.2												Sparrow et al., 1995, Fairbanks
M. sativa	1988	44	88	2.1												Sparrow et al., 1995, Delta Junction
M. sativa	1989	23	71	1.9												Sparrow et al., 1995, Delta Junction
M. sativa	1981				242	70	12.1	319	80	13.6						Wivstad et al., 1987
M. sativa	1990	83	59	4.5	156	74	9.3	187	75	10.5						Walley et al., 1996, monoculture
M. sativa	1990	62	62	3.3	138	86	6.4	213	89	8.7						Walley et al., 1996, mixture
M. sativa	1983	93	69		258	81		227	79							Burity et al., 1989
M. sativa	1982	75	60	5.2	250	75	11.0	220	75	10.0	160	85	4.5			Heichel and Henjum, 1991

[a] *Trifolium* fields are often undersown in cereal crops, which partly explains why legume production and N_2 fixation is not always measured during the sowing year.
[b] Production years are 1997 and 1998.
[c] Production years are 1995 and 1996.
[d] Fertilized with 210 kg N ha^{-1} $year^{-1}$.

common to measure total root biomass. An absolute measure of total N_2 fixation is hard to achieve even if all root N can be measured because N is also lost from plants through root leakage and root breakdown (Witty, 1983a). However, as long as it is taken into account that a substantial part of fixed N_2 is not recorded in field measurements, measurements of aboveground N_2 fixation are often the only realistic measures to get. Most values of N_2 fixation per ha and year presented in Tables 1–5 and 7–9 are underestimates, since few studies include fixed N in roots.

Variation between sites

Comparisons of N_2 fixation activities at different sites are often confounded by different experimental designs, different plant varieties, fertilization, etc. However, Nesheim and Øyen (1994) and Sparrow et al. (1995) performed comparable experiments at geographically different sites. During two production years and with different fertilizer regimes in Norway, the amount of N_2 fixation was always higher at 59°N than at 67°N (Nesheim and Øyen, 1994). However, many factors apart from latitude (clover proportion in stands, local *Rhizobiom* strains, varieties of clover, soils) could contribute to this difference (Nesheim and Øyen, 1994). In Alaska, Sparrow et al. (1995) measured N_2 fixation in seven legumes at Fairbanks (64.5°N) and at Delta Junction (63.5°N) during two seasons. N_2 fixation and plant DM production in *T. pratense* and *M. sativa* were considerably higher at Fairbanks. In experiments performed close to each other variation in N_2 fixation has been ascribed to different soil types (Goodman, 1988; Munro and Davies, 1974; Rice, 1980). When data on N_2 fixation from 36 studies are plotted against latitude there is no simple correlation between activity and latitude (Figure 8). It is concluded that any large-scale effects of geographic location are secondary to local effects, such as soil type, microclimate, management, plant variety and *Rhizobium* strain, which may have large influence on N_2 fixation. It is likely that the perennial forage legumes discussd in this review, partly due to successful breeding, are well adapted to low temperatures and can take advantage of the long days in northern areas.

Variation due to grazing or cutting management

In almost all studies with *T. pratense* and *M. sativa* the experiments are performed with cutting management. On the other hand, about one-third of the studies on *T.*

Table 8. Variation between species and varieties in N_2 fixation (Nfix, kg N ha^{-1} year^{-1}), Ndfa (%) and legume dry matter yield (DM, ton ha^{-1} year^{-1}). < 20 kg N fertilization ha^{-1} year^{-1}, ID, except when noted differently

M. sativa			*T. pratense*			*T. repens*						
Nfix	Ndfa	DM	Nfix	Ndfa	DM	Nfix	Ndfa	DM	Nfix	Ndfa	DM	Reference
58	49	5.0	121	61	9.6							Sparrow et al., 1995, Fairbanks, year 1
63	56	5.2	41	43	4.2							Sparrow et al., 1995, Fairbanks, year 2
44	88	2.1	76	85	3.8							Sparrow et al., 1995, D.J., year 1
23	71	1.9	33	85	2.1							Sparrow et al., 1995, D.J., year 2
			165	92	6.0	227	83	7.3				Boller and Nösberger, 1987, sowing year
			49	81	1.5	83	81	2.3				Boller and Nösberger, 1987, sowing year
			373	84	14.3	268	78	8.9				Boller and Nösberger, 1987, prod year
			307	85	12.1	283	77	9.8				Boller and Nösberger, 1987, prod year
75	60	5.2	8	40	2.5	1	40	0.7				Heichel and Henjum, 1991, year 1
250	75	11.0	150	70	8.5	20	75	0.9				Heichel and Henjum, 1991, year 2
220	75	10.0	80	90	3.0	15	85	0.3				Heichel and Henjum, 1991, year 3
160	85	4.5	25	93	0.5	1	93					Heichel and Henjum, 1991, year 4
			177		5.6	103		3.3				Ledgard et al., 1990, year 1
			162	63	5.4	269	73	7.4				Ledgard et al., 1990, year 2
T. pratense, var. 1			*T. pratense*, var. 2			*T. pratense*, var. 3			*T. pratense*, var. 4			
Nfix	Ndfa	DM	Nfix	Ndfa	DM	Nfix	Ndfa	DM	Nfix	Ndfa	DM	
145	80	6.7	215	85	8.4							Boller and Nösberger, 1994, 0 N
76	73	4.0	123	79	5.3							Boller and Nösberger, 1994, 150 kg N
159	97	4.1	153	96	4.4	140	96	2.9	158	97	4.5	Farnham and George, 1993, year 1
117	98	4.6	107	98	4.1	76	98	2.1	73	98	3.1	Farnham and George, 1993, year 2
259	91	9.4	202	93	8.2	271	92	7.5	251	94	9.3	Farnham and George, 1994, year 1, 3-cut
174	97	5.0	189	97	4.9	195	96	5.3	184	96	4.7	Farnham and George, 1994, year 1, 6-cut
137	94	6.0	165	93	6.1	77	96	2.3	149	94	5.8	Farnham and George, 1994, year 2, 3-cut
143	95	4.7	134	95	4.7	66	94	2.0	118	91	4.2	Farnham and George, 1994, year 2, 6-cut
T. repens, var. 1			*T. repens*, var. 2			*T. repens*, var. 3			*T. repens*, var. 4			
Nfix	Ndfa	DM	Nfix	Ndfa	DM	Nfix	Ndfa	DM	Nfix	Ndfa	DM	
545	93[a]	11.1	465	92[a]	8.7	440	92[a]	8.0				Elgersma and Hassink, 1997, year 1, ND
470	95[a]	9.0	425	94[a]	7.2	280	92[a]	4.3				Elgersma and Hassink, 1997, year 2, ND
307	92[a]	6.0	217	89[a]	3.5	150	85[a]	2.2				Elgersma and Hassink, 1997, year 3, ND
407	94[a]	7.7	420	94[a]	7.8	309	92[a]	5.5				Elgersma and Hassink, 1997, year 4, ND
445	94[a]	8.4	415	94[a]	7.4	320	92[a]	5.7				Elgersma et al., 1998, year 1, ND
271	91[a]	5.3	217	89[a]	3.4	220	89[a]	3.8				Elgersma et al., 1998, year 2, ND
82		3.1	119		3.7	110		3.5	112		3.3	Ledgard et al., 1990, year 1
224	68	6.5	285	75	7.7	291	77	7.6	278	74	8.3	Ledgard et al., 1990, year 2

[a]Calculated from data in original paper as Ndfa = Nfix / (total clover N yield).

repens are grazing experiments (Tables 1, 2 and 4, Figure 8). There is no difference in Nfix between cutting and grazing experiments (Figure 8). On the other hand, Ndfa in grazed *T. repens* plots is significantly higher than in cutting experiments, and similar to Ndfa in *T. repens*/grass mixtures ($\text{Ndfa}_{\text{grazed}} = 81$, $\text{Ndfa}_{\text{cut}} = 70$, $P < 0.05$). Accordingly, the model for calculating Nfix in *T. repens* using grazing data is different from the model based on cutting data ($\text{Nfix}_{\text{grazed}} = 0.033 \cdot \text{DM} + 25.8$, $R^2 = 0.76$; $\text{Nfix}_{\text{cut}} = 0.025 \cdot \text{DM} + 36.5$, $R^2 = 0.59$; cf. models in Table 6).

Since legume biomass production has to be known in order to obtain a value of kg N fixed per ha and year, Nfix data from grazing studies are derived from plots that were excluded from grazing animals or from pastures that were rotationally grazed (i.e., grazing the pasture at limited time periods and allowing plant biomass to accumulate between these periods). Thus, the treatments resulting in the grazing data presented in

Table 9. Variation in N_2 fixation (kg N ha^{-1} year^{-1}) due to method used within the same study

ID	NA	ND	ND/ID	Species	Reference
N in grass monoculture subtracted from total N in legume–grass mixture for ND					
83		113	1.36	*T. pratense*	Nesheim and Øyen, 1994
162		219	1.35	*T. pratense*	Nesheim and Øyen, 1994
173		204	1.18	*T. repens*	Boller and Nösberger, 1987
255		299	1.17	*T. pratense*	Boller and Nösberger, 1987
258		295	1.14	*T. pratense*	Nesheim and Øyen, 1994
325		357	1.1	*T. pratense*	Boller and Nösberger, 1987
122		132	1.08	*T. pratense*	Boller and Nösberger, 1987
173		185	1.07	*T. pratense*	Nesheim and Øyen, 1994
161		171	1.06	*T. pratense, T. repens*	Høgh-Jensen and Kristensen, 1995
157		164	1.04	*T. pratense, T. repens*	Høgh-Jensen and Kristensen, 1995
161	138[a], 166[b]			*T. pratense, T. repens*	Høgh-Jensen and Schjørring, 1994
157	118[a], 142[b]			*T. pratense, T. repens*	Høgh-Jensen and Schjørring, 1994
N in grass monoculture subtracted from N in legume monoculture for ND, except when noted differently					
121		129	1.07	*T. pratense*	Sparrow et al., 1995
76		72	0.95	*T. pratense*	Sparrow et al., 1995
84		79	0.94	*M. sativa*	Mårtensson and Ljunggren, 1984
58		52	0.9	*M. sativa*	Sparrow et al., 1995
44		34	0.77	*M. sativa*	Sparrow et al., 1995
171		126	0.74	*T. repens*	McNeill and Wood, 1990
56		40	0.71	*T. pratense*	Witty, 1983a
63		43	0.68	*M. sativa*	Sparrow et al., 1995
23		15	0.65	*M. sativa*	Sparrow et al., 1995
41		26	0.63	*T. pratense*	Sparrow et al., 1995
33		20	0.61	*T. pratense*	Sparrow et al., 1995
155		84	0.54	*T. repens*	McNeill and Wood, 1990[c]

[a] *B* value (isotopic discrimination during N_2 fixation) set to 0.
[b] *B* value set to 1.2, calculated from data in original paper.
[c] N in the grass part of a clover–grass mixture subtracted from N in the clover part of the mixture for ND.

Figure 8 are combinations of cutting and grazing management. Nitrogenase activity has been measured in situ in trials with continuous grazing using the ARA technique (Masterson and Murphy, 1976; Parsons et al., 1991) but, as stated in the methods section, ARA can not be recommended for N_2 fixation estimates in field.

In *T. pratense* and *T. repens* grown together in mixture with grass, Nfix did not differ between cutting frequency (3 or 6 cuts year^{-1}, Høgh-Jensen and Kristensen, 1995; Høgh-Jensen and Schjørring, 1994). *Trifolium pratense* in mixture with grass fixed slightly more N when cut 3 times as compared to 6 times per year, but the difference was not statistically significant (Farnham and George, 1994). In other studies, frequent defoliation in spring did increase Nfix in *T. repens* pastures (Ledgard et al., 1992). The effect of grazing on N_2 fixation and legume production also depends on which animals that graze the sward (Ledgard et al. 1992).

Conclusions

Perennial forage legumes take a large proportion of their N from N_2 fixation, especially when intercropped with grasses. Consequently, there is good correlation between N_2 fixation per ha and year and legume dry matter yield. This suggests that managements favoring legume productivity should ensure high N_2 fixation.

Choice and use of method may influence the estimated value of N_2 fixation. For seasonal or annual measurements in field, the isotope-based methods are recommended as long as isotopic discrimination is considered (for NA) and appropriate reference crops

Table 10. Variation in the estimation of N_2 fixation (kg N ha^{-1} $year^{-1}$) due to harvested plant parts

Fixed N above cutting height, percent of whole plants	Species	Basis for calculation of fixed N in shoots[a]	Basis for calculation of fixed N in roots	Reference
94	*T. pratense, T. repens, M. sativa, L. corniculatus*	Seasonal N yield	Seasonal N increment in perennial sward	Heichel & Henjum, 1991
87[b]	*T. repens*	Annual N yield	Existing root N in perennial sward	Ledgard et al., 1987
81[b]	*T. repens, T. subterraneum*	Annual N yield	Existing root N in perennial sward	Ledgard et al., 1987
78[b]	*T. repens*	Seasonal N yield	Seasonal N yield	Kumar & Goh, 2000
44–67[b]	*T. repens*	Seasonal N yield	Existing root N in perennial sward	Goodman, 1988
43[b]	*M. sativa*	Seasonal N yield	Existing root N (after 3 years)	Walley et al., 1996
40[b]	*M. sativa*	Seasonal N yield	Seasonal N yield (sowing year)	Walley et al., 1996
36[b]	*M. sativa*	Seasonal N yield	Existing root N (after 2 years)	Walley et al., 1996

[a]Studies performed at locations where the production ceases during winter refer to seasonal yield, while studies performed in areas with continuous production over the year use the term annual yield.
[b]Recalculated from data in the original paper.

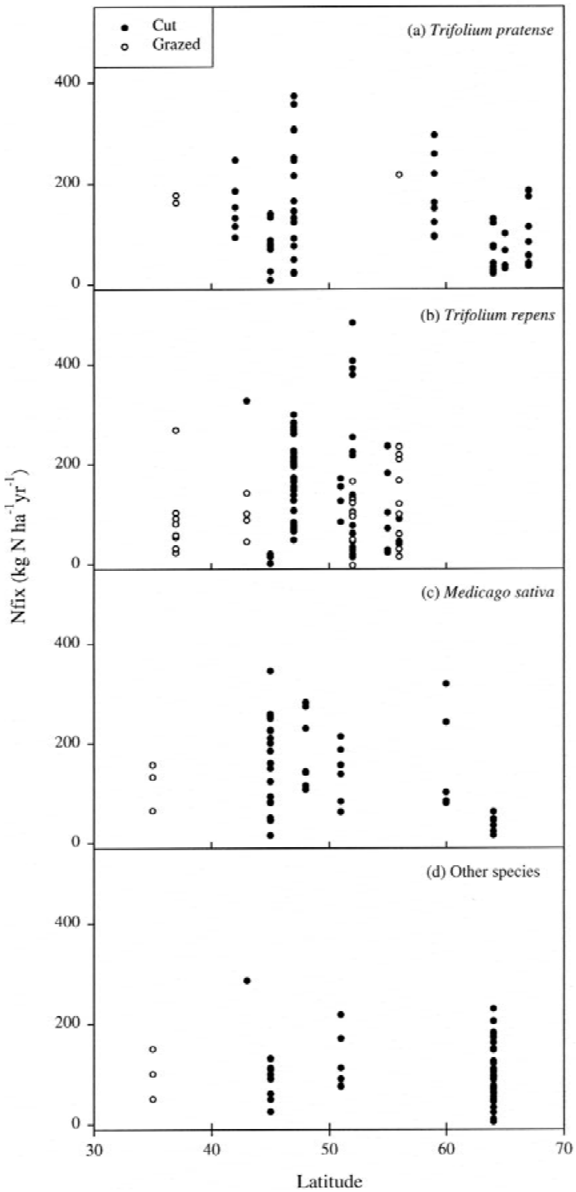

Figure 8. N_2 fixation related to latitude. Further details, see legend of Figure 1.

are used. For rough estimations of N_2 fixation, equations based on legume dry matter yield can be used.

Due to their large dependence on N_2 fixation, perennial forage legumes are very valuable in reducing the use of industrial fertilizers. By sparing soil N and transfer of fixed N to soil these plants contribute significantly to the N supply also to other crops.

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