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# A plant nitrophily index based on plant leaf area response to soil nitrogen availability

Delphine Moreau · Guillaume Milard · Nicolas Munier-Jolain

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**Abstract** This article reports a new experimental method to measure plant nitrophily. Knowledge of the nitrophily of plant species has many potential applications such as studying the long-term evolution of flora and designing nitrogen management strategies in cropping systems. Plant nitrophily is commonly measured by the Ellenberg N score based on the natural occurrence of species along soil nitrogen gradients. The Ellenberg N score is known for species from restricted geographical areas representing a small proportion of total plant species diversity. In addition, measuring Ellenberg N score is not convenient. We propose a new definition of plant nitrophily referring to plant leaf area response to nitrogen availability. We compared habitat-based and response-based values of nitrophily to design a simple method to estimate a plant nitrophily index (NI). Eleven monocotyledonous and dicotyledonous plant species were grown in a greenhouse experiment at two levels of soil nitrogen. Nine species were weeds covering the range of the Ellenberg N score. Two crop species with unknown nitrophily, wheat and oilseed rape, were studied to illustrate our method. Plant leaf area was measured at one date for each species  $\times$  nitrogen treatment combination. A NI was calculated as the ratio of leaf area at high nitrogen to leaf area at low nitrogen. Our results show for weeds a high interspecific diversity of the NI, ranging from 1.0 to 4.2. The NI was strongly and positively correlated to Ellenberg N score, with  $R^2$  of 0.73. The more nitrophilic a species according to habitat preferences, the more leaf area responded to increasing nitrogen supply. This is the first time that a quantitative relationship is found between Ellenberg N score and a growth variable measured non-destructively for both monocotyledonous and dicotyledonous species. Therefore, calculating the response of

leaf area to nitrogen supply provides a new simple and non-destructive method that can be used for the assessment of a NI of any species. As an illustration of the method, a NI was estimated for new species. It was at 2.1 and 4.5 for wheat and oilseed rape, respectively, indicating that they were respectively moderately and highly nitrophilic. This method will help to assess the relative nitrophily of weeds vs. crops. Such knowledge could be used to design nitrogen management strategies promoting crop growth but not weed growth, thus reducing the use of herbicides.

**Keywords** Plant species · Nitrophily · Oligotrophy · Leaf area · Nitrogen · Ellenberg N score · Weed

## 1 Introduction

For plant species, nitrogen (N) availability and absorption are major determinants of plant growth and seed production, with consequences for survival of spontaneous species and for crop yield (Uhart and Andrade 1995; Lawlor 2002; Burkle and Irwin 2010). The impact of N nutrition is mainly on meristematic and photosynthetic activities and therefore on plant leaf area expansion and carbon acquisition (Snyder and Bunce 1983; Sinclair and Horie 1989; Vos and Biemond 1992; Vos and van der Putten 1998). Plant species differ in their N requirements, a reason why they naturally occupy different types of environments. Nitrophily can be defined either with the point of view of plant ecology, considering the species habitats, or with an ecophysiological point of view, considering the plant physiological response to soil N availability. According to the point of view of plant ecology, “nitrophilic” species are observed mainly in sites with high soil N availability, especially nitrates, and are classically opposed to “oligotrophic” species that are mostly found in environments with low N in soils (Boulard 1998). Each

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plant species can be classified according to its nitrophily index (NI), ranging from the most oligotrophic to the most nitrophilic ones. The NI of plant species is an ecological indicator that is useful to analyze the long-term evolution of the floristic composition of plant communities. Nitrophily was shown to explain a large part of differences in plant communities along soil N gradients both in natural and in intensively cultivated ecosystems and also to explain changes in weed communities in arable fields during the intensification of agriculture in the past decades (Smart et al. 2003; Willi et al. 2005; Fried et al. 2009). Knowing plant species nitrophily can also be useful to better understand the competition among species in plurispecific communities. Indeed, the intensity of competition between two species and the consequences for the growth of competing plants can be strongly affected by complex interaction between the N availability and the relative nitrophily of the species. Indeed, the most nitrophilic species are classically found to be the most competitive ones (Tilman 1987; Padgett and Allen 1999; Valantin-Morison et al. 2003), but the ranking of competitive ability might depend on N availability (Iqbal and Wright 1997).

Information on the NI of plant species is currently extracted from the ecological database of Ellenberg (1974). From observations of frequency and abundance of plant species along gradients of soil N richness, Ellenberg (1974) developed the Ellenberg N score ranking species on a scale from 1 for the most oligotrophic species to 9 for the most nitrophilic ones. He classified about 2,000 species from the western part of Central Europe. However, the scores referenced in the Ellenberg database should be used with caution outside the region where they were defined (Andel and Nelissen 1979; Hill et al. 2000). As a result, enlarging the domain of validity of Ellenberg N scores to new geographical areas and/or new plant species has become necessary (Hill et al. 2000). The method used by Ellenberg, observing flora on soils covering a large range of soil N richness, is extremely time consuming and requires a strong botanical expertise. Hill et al. (2000) proposed to extend Ellenberg's indicator values to a new geographical area using an algorithmic approach. However, their method requires a large database based on flora surveys, and the "success of prediction—of their method—was only moderate." Similar drawbacks were identified for the method of Diekmann et al. (1998), based on flora surveys and measurements of the mineralization rates of N in the soils where species are present. Alternatively, Fichtner and Schulze (1992) identified a strong and positive correlation between the Ellenberg N scores and the response of relative growth rate to soil N supply for a range of dicotyledonous species. The response of relative growth rate to soil N supply can therefore be used to quantify nitrophily with the point of view of the plant ecophysiological. This method avoids

performing flora surveys, but requires measurements of plant biomass at different dates during the plant cycle, which is time consuming. Moreover, as measuring plant biomass is destructive, it requires growing a relatively high number of plants per species, which can be impossible when a limited number of seeds are available. Finally, plant biomass is the most frequently used growth variable to differentiate nitrophilic from oligotrophic species with the point of view of plant physiology (Chapin 1980; Padgett and Allen 1999), but considering plant leaf area could be more appropriate. Indeed, the first physiological function affected by N starvation is plant leaf area expansion, and only thereafter, photosynthesis per unit leaf area is reduced (Grindlay 1997). Thus, plant leaf area appears as a better indicator of plant N nutrition than plant biomass. Moreover, it is compatible with non-destructive measurements combining plant photography and image analysis now feasible with automated phenotyping platforms (e.g., Granier et al. 2006; Salon 2012). Here, our objective was to determine whether measuring the response of plant leaf area to soil N supply provides a good method to calculate a "plant response-based" NI, considering both dicotyledonous and monocotyledonous species, and to consider the consistency with the "habitat-based" Ellenberg N index. We considered 11 species: nine of them were contrasted for their Ellenberg N score, and the two others were suspected to be contrasted for their NI, but their Ellenberg N index is unknown. Each species was grown in a greenhouse experiment at two contrasted conditions of soil N supply. Plant leaf area was measured non-destructively, and the correlation between Ellenberg N scores and the response of plant leaf area to soil N was analyzed. A NI was calculated for the two species with unknown Ellenberg score, and the results were compared with those in the literature.

## 2 Materials and methods

### 2.1 Plant material and cultural conditions

A greenhouse experiment was conducted in Dijon (France) using a panel of 11 monocotyledonous and dicotyledonous annual plant species (Table 1). Nine weed species were chosen to cover the range of Ellenberg N scores. Two additional crop species were included: oilseed rape that is commonly considered the most nitrophilic crop species in temperate cropping systems and wheat that is consequently considered to be less nitrophilic than oilseed rape (Fig. 1). Seeds were germinated in an incubator with a photoperiod of 12 h and day and night temperatures of 25 and 15 °C, respectively, except for *Polygonum lapathifolium* (30 and 20 °C). Thereafter, germinated seeds were sown in a glass-house into 7-L pots filled with a solid, inert, and draining substrate (1:1 mixture of expanded clay and attapulgitite).

**Table 1** Characteristics of the studied species. Ellenberg N score indicates the nitrophily status of plants

Ellenberg N score	Species (cultivar for crops)	Type
1	<i>Vulpia myuros</i> (L.) Gmelin	Monocotyledonous
2	<i>Teucrium botrys</i> L.	Dicotyledonous
2	<i>Thlaspi perfoliatum</i> L.	Dicotyledonous
3	<i>Bromus hordeaceus</i> L.	Monocotyledonous
4	<i>Geranium molle</i> L.	Dicotyledonous
6	<i>Alopecurus myosuroides</i> Huds.	Monocotyledonous
8	<i>Echinochloa crus-galli</i> (L.) Beauv.	Monocotyledonous
8	<i>Matricaria perforata</i> Mérat.	Dicotyledonous
8	<i>Polygonum lapathifolium</i> L.	Dicotyledonous
ND	<i>Brassica napus</i> (oilseed rape cv Cador)	Dicotyledonous
ND	<i>Triticum aestivum</i> (wheat cv Caphorn)	Monocotyledonous

ND not determined

During 53 days, species were grown under two N treatments: a low nitrate concentration (2.5 mM during the first 33 days and 0.625 mM thereafter) and a high nitrate concentration (10.5 mM throughout the experiment). Each nutrient solution was made up of N, phosphorous, potassium, and oligoelements (Moreau et al. 2008) and was provided by automatic watering several times a day at a frequency allowing the pots to drain in order to avoid the accumulation of ions in the substrate. Air temperature (PT100 sensors; Pyro-Contrôle, Vaulx-en-Velin, France) and incident photosynthetically active radiation (PAR; silicium sensors; Solems, Palaiseau, France) measurements were made at 600-s intervals and stored in a data logger (DL2e; Delta-T Devices, Cambridge, England). Air temperature was  $17.4 \pm 2.5$  °C, and incident PAR was  $26.6 \pm 16.5$  mol m<sup>-2</sup> jour<sup>-1</sup>.



**Fig. 1** Wheat field with weed species (*Centaurea*, *Matricaria*, and *Papaver* species). The intensity of competition between two species in plant communities and the consequences for the growth of competing plants can be strongly affected by complex interactions between soil N availability and the relative nitrophily of plant species. © INRA Gilles Louvot

## 2.2 Plant measurements

At 53 days after sowing, plant leaf area was estimated non-destructively on seven plants per species  $\times$  N treatment combination. Pictures of individual plants were taken with two cameras (top view and side view). They were analyzed using semi-automatic image analysis (Moreau et al. 2009; Mathey et al. 2011) to estimate for each plant a projected top leaf area and a projected side leaf area.

## 2.3 Statistical analyses

ANOVAs were performed to analyze the effects of species and N supply on plant leaf area, and linear regressions were fitted between Ellenberg N scores and the response of plant leaf area to soil N supply, using Statgraphics Plus 16.0.07 for Windows (Statistical Graphics Corp., Rockville, MD). Statistical differences were judged at  $\alpha=0.05$ .

## 3 Results and discussion

### 3.1 Plant leaf area

Both for top and side leaf areas, the species effect, the N treatment effect, and the species  $\times$  N treatment interactions were all significant ( $P<0.001$ ; Table 2). *Vulpia myuros* produced the smallest top leaf area, while *P. lapathifolium* produced the highest (Fig. 2). Moreover, the leaf area of *V. myuros* was unresponsive to soil N whereas the leaf area of *P. lapathifolium* was multiplied by a factor of approximately 3 with high soil N supply. Finally, very different absolute values of plant leaf area at high soil N were obtained for species with identical Ellenberg N scores. This was observed for *Echinochloa crus-galli*, *Matricaria inodorum*, and *P. lapathifolium* whose Ellenberg N scores are at

**Table 2** Top and side leaf areas of the studied species in two conditions of soil N availability in a greenhouse experiment. Nitrophily index calculated as the ratio of top leaf area at high soil N to top leaf area at low soil N. Values are mean  $\pm$  standard error. Weed species are ranked according to their Ellenberg N score (from the more

oligotrophic to the more nitrophilic species; see Table 1). Note the high interspecific diversity of plant leaf area in both soil N treatments and of the response of plant leaf area to soil N supply. This latter is reflected by the nitrophily index which accounts for the response of top leaf area to increasing soil N

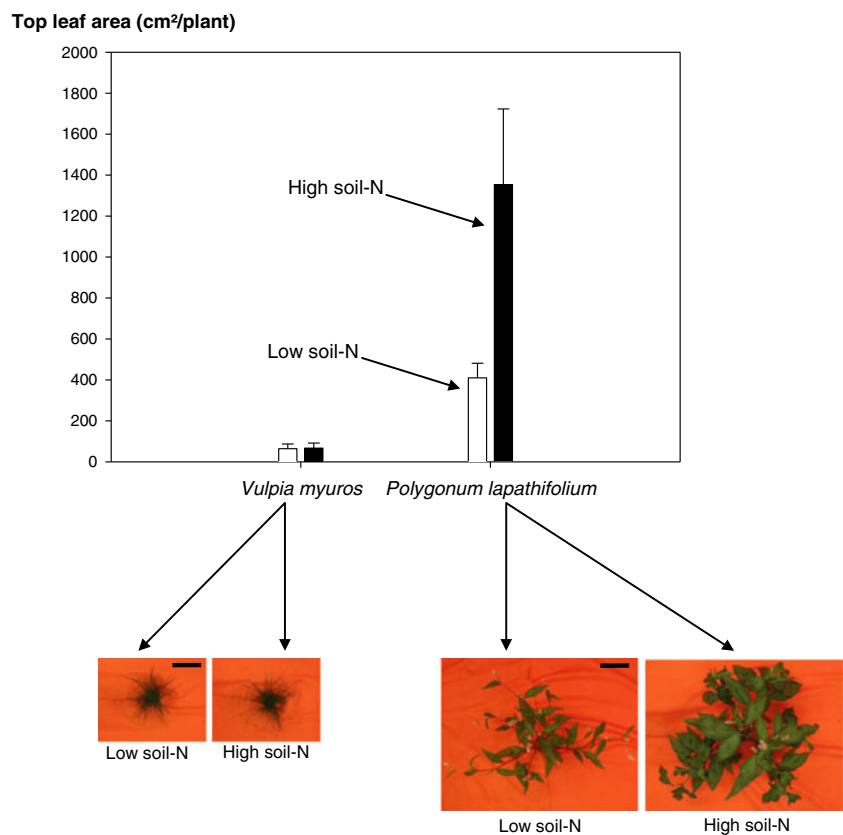
Species	Top leaf area (cm <sup>2</sup> per plant)		Side leaf area (cm <sup>2</sup> per plant)		Nitrophily index
	At low soil N	At high soil N	At low soil N	At high soil N	
<i>Vulpia myuros</i>	64 $\pm$ 23	67 $\pm$ 24	36 $\pm$ 9	41 $\pm$ 14	1.04 $\pm$ 0.53
<i>Teucrium botrys</i>	138 $\pm$ 41	172 $\pm$ 44	76 $\pm$ 19	92 $\pm$ 23	1.25 $\pm$ 0.49
<i>Thlaspi perfoliatum</i>	69 $\pm$ 10	108 $\pm$ 18	35 $\pm$ 6	52 $\pm$ 12	1.57 $\pm$ 0.35
<i>Bromus hordeaceus</i>	137 $\pm$ 39	254 $\pm$ 78	82 $\pm$ 23	147 $\pm$ 47	1.86 $\pm$ 0.77
<i>Geranium molle</i>	169 $\pm$ 18	259 $\pm$ 114	70 $\pm$ 9	145 $\pm$ 114	1.53 $\pm$ 0.70
<i>Alopecurus myosuroides</i>	136 $\pm$ 65	348 $\pm$ 97	88 $\pm$ 44	212 $\pm$ 61	2.57 $\pm$ 1.42
<i>Echinochloa crus-galli</i>	187 $\pm$ 52	781 $\pm$ 334	141 $\pm$ 47	412 $\pm$ 133	4.19 $\pm$ 2.14
<i>Matricaria perforata</i>	152 $\pm$ 29	330 $\pm$ 117	128 $\pm$ 22	263 $\pm$ 73	2.18 $\pm$ 0.88
<i>Polygonum lapathifolium</i>	410 $\pm$ 71	1354 $\pm$ 369	175 $\pm$ 19	579 $\pm$ 168	3.30 $\pm$ 1.07
Oilseed rape	326 $\pm$ 50	1472 $\pm$ 254	177 $\pm$ 49	655 $\pm$ 88	4.52 $\pm$ 1.05
Wheat	248 $\pm$ 29	513 $\pm$ 174	208 $\pm$ 28	375 $\pm$ 104	2.07 $\pm$ 0.74

8 (Table 2), indicating different leaf growth potential among nitrophilic species. Thus, growing a panel of species covering the range of Ellenberg N scores at two contrasted conditions of soil N supply generated a wide range of plant leaf area.

### 3.2 Correlation between Ellenberg N score and the response of top leaf area to soil N

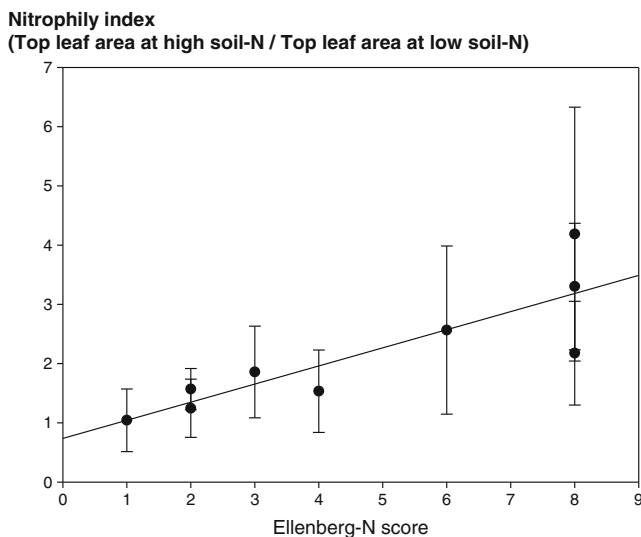
For the nine weed species with known Ellenberg N scores, the ratio of top leaf area at high soil N to top leaf area at low

**Fig. 2** Top leaf area at the end of the greenhouse experiment for two contrasted species at two conditions of soil N availability. Values are mean  $\pm$  standard error. Examples of pictures used to estimate top leaf area are provided (horizontal bars indicate 10 cm). The leaf area of *V. myuros* was unresponsive to soil N whereas the leaf area of *P. lapathifolium* was multiplied by a factor of approximately 3 with high soil N supply. This result shows a high interspecific variability related to the response of plant leaf area to soil N supply





soil N was calculated to account for the response of top leaf area to increasing soil N (Table 2). It was related to Ellenberg N scores (Fig. 3). A strong positive and linear correlation was found between both variables ( $R^2=0.73$ ;  $P=0.0035$ ) including both monocotyledonous and dicotyledonous species. The more nitrophilic a species according to Ellenberg N score, the more it responded to increasing soil N supply by increasing top leaf area. This finding shows that even at low soil N, a large part of the N requirements of oligotrophic species are fulfilled so that enhancing soil N availability only slightly increases plant leaf area (Chapin 1980). To our knowledge, studies showing a quantitative relationship between Ellenberg N scores and a plant growth variable are still very scarce. Only Fichtner and Schulze (1992) showed such a relationship for dicotyledonous species only and using destructive measurements of plant biomass at several dates during the plant cycle. The novelty of our approach is that a single relationship is valid for very diverse species and estimable by simple non-destructive measurements performed at a single date during the plant cycle. Thus, calculating the ratio of top leaf area at high soil N to top leaf area at low soil N was found as a new method to calculate the NI of plant species.



**Fig. 3** Relationship between nitrophily index and Ellenberg N scores for nine plant species. Nitrophily index is calculated for each species as the ratio between top leaf area at high soil N and top leaf area at low N. Values are mean  $\pm$  standard error. The regression equation is  $y=0.3059x+0.7377$  ( $R^2=0.73$ ). Note the increase of the nitrophily index with increasing Ellenberg N scores according to a unique relationship including monocotyledonous and dicotyledonous species. This good correlation shows that the preference of nitrophilic species for N-rich habitats is linked to their ability to respond to increased soil N by extending leaf area. Measuring the response of plant leaf area to soil N is shown as a convenient method to measure rapidly the nitrophily index of plant species

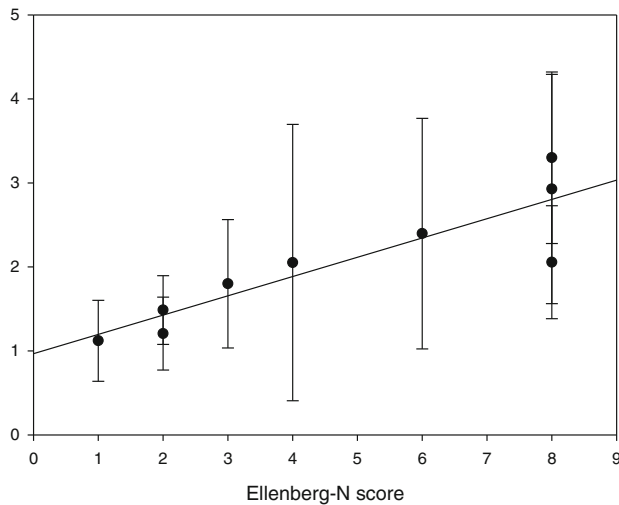
### 3.3 Correlation between Ellenberg N score and the response of side leaf area to soil N

Measuring plant leaf area with a top view is reasonably the most relevant method to account for the plant leaf area which is exposed to light in plant communities. However, to test the validity of our method, a similar analysis was performed considering side leaf area instead of top leaf area (Fig. 4). Again, a strong positive and linear correlation was found between Ellenberg N scores and the ratio of side leaf area at high soil N to side leaf area at low soil N ( $R^2=0.79$ ;  $P=0.0014$ ). The same conclusion as above could be drawn, confirming with a different method the existence of a unique relationship between Ellenberg N score and the response of plant leaf area to soil N. The relationships between Ellenberg N score and the response of top or side leaf area to soil N produce an ecophysiological explanation of the decline in oligotrophic species in N rich soils (Fried et al. 2009). Their absence is probably explained by their exclusion by competition with nitrophilic species which have a high leaf area and therefore (1) a strong light interception and associated photosynthesis and (2) a strong capacity to shade neighboring plants. Our findings highlight the importance of competition for light as a mechanism contributing to plant diversity loss in agreement with a classically observed switch from belowground competition for nutrients to aboveground competition for light when soil resources are enhanced (Tilman 1987; Hautier et al. 2009).

### 3.4 Application to new species

Our experiment included wheat and oilseed rape, two crop species with unknown Ellenberg N scores. Their NI was calculated as the ratio of top leaf area at high soil N to top leaf area at low soil N: it was at 2.1 and 4.5 for wheat and oilseed rape, respectively. Using our new NI vs. Ellenberg N score relationship, we estimated the Ellenberg N score for each of the two species from the ratio of top leaf area at high soil N to top leaf area at low soil N for each of the two species (Fig. 5). Estimated Ellenberg N scores were 4.4 and 12.4 for wheat and oilseed rape, respectively. Thus, wheat was found to be moderately nitrophilic and oilseed rape very nitrophilic, compared to the range of variations for the 11 studied species (Fig. 2). The strong nitrophily observed for oilseed rape is consistent with Valantin-Morison et al. (2003) showing that the ability of oilseed rape canopies to suppress weeds increased with soil N availability. The moderate nitrophily of wheat is in agreement with its relatively low capacity to increase its competitiveness toward weeds in response to increase soil N (Iqbal and Wright 1997). Altogether, these results validate our method to estimate the NI of any annual species and illustrate how it can be used to rank species according to their NI.

Side leaf area at high soil-N / Side leaf area at low soil-N

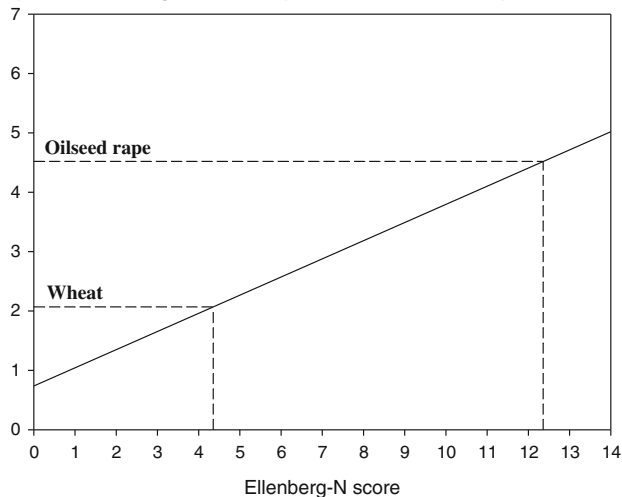


**Fig. 4** Relationship between the ratio of side leaf area at high soil N and side leaf area at low N for nine plant species. Values are mean  $\pm$  standard error. The regression equation is  $y=0.2295x+0.9671$  ( $R^2=0.79$ ). Note the increase of the ratio of side leaf area at high soil N to side leaf area at low soil N with increasing Ellenberg N score. This correlation confirms the existence of a unique relationship between Ellenberg N score and the response of plant leaf area to soil N

### 3.5 Methodology and practical recommendations for a further use of the method

Estimating a NI for a new species requires (1) carrying out an experiment at two contrasted conditions of soil N including

**Nitrophily index**  
(Top leaf area at high soil-N / Top leaf area at low soil-N)



**Fig. 5** Assessment of an Ellenberg N score for oilseed rape and wheat using their nitrophily index and the regression equation obtained for the nine species for which Ellenberg N score is known. The regression equation comes from Fig. 3. With a calculated Ellenberg N score of 4.4 and 12.4 for wheat and oilseed rape, respectively, wheat was found to be moderately nitrophilic and oilseed rape very nitrophilic in agreement with the literature. This figure illustrates how our method can be used to assess an Ellenberg N score for new species

both species for which Ellenberg N score is known that are considered as “control” and new species for which the NI and Ellenberg N scores are to be estimated and (2) measuring plant leaf area for all species at a given date. A NI index can then be calculated for each species as the ratio of top leaf area at high soil N to top leaf area at low soil N. The correct ranking of control species can be checked to validate the experiment. Nitrophily estimated with this method can be expressed using the scale of Ellenberg N score by transforming NI into Ellenberg N value using the linear relationship observed on species from Ellenberg's database. Two recommendations are essential for a successful estimation of the Ellenberg score for new species. Firstly, the equation of the regression between Ellenberg N score and the response of top leaf area to soil N varies with the experimental conditions (light, photoperiod, date of measurements of plant leaf area, N availability in both treatments). As a result, the equation is valid for a given experiment and cannot be used to estimate the Ellenberg N score of species grown in another experiment. Secondly, the effect of the N treatments requires time to be detected. Thus, plant leaf area measurements should ideally be performed lately but before the beginning of the flowering stage and not after as the rules of allocation of carbon toward leaves are known to be modified during reproductive growth (Rajcan and Tollenaar 1999).

### 4 Conclusion

This paper demonstrates a convergence between the habitat-based definition of nitrophily from plant ecology and the response-based definition of nitrophily from plant ecophysiology. The low capacity of oligotrophic species to increase their plant leaf area in response to increasing soil N produces an ecophysiological explanation of the decline of these species in N-rich soils. From a practical point of view, this paper provides a new and simple method for estimating nitrophily of monocotyledonous and dicotyledonous species. It is based on a single experiment in controlled conditions with two contrasted levels of soil N supply and on non-destructive measurements of plant leaf area at a single date. This method is less time consuming than performing flora surveys along N gradients, as performed by Ellenberg (1974). As it uses non-destructive measurements, only a small number of plants per species are needed. As a result, it makes it possible to estimate nitrophily of any species even when few seeds are available. Nitrophily can be estimated using this method from any experiment designed for any other purpose as soon as the experimental design includes contrasted N nutrition levels. Concrete applications of the method are envisaged. In addition to the classical utilization of NI for analyzing the evolution of flora at the long term and for analyzing competitive relationships within plant communities, this should help to estimate relative crop/weed

nitrophily, hence providing guidelines to design N supply management strategies in cropping systems with low reliance on herbicides. For highly nitrophilic crops such as oilseed rape, with an increased ability to compete with weeds with increased soil N, providing an optimal level of N fertilization in accordance with practical recommendations seems to be the best way to favor crop growth at the expense of weeds. So, there is probably little to be expected from a modulation of the quantity of N fertilizer as a lever to reduce the use of herbicides. By contrast, for moderately nitrophilic crops such as wheat, it seems interesting to analyze the benefit that could be derived from a slight reduction of the quantity of N fertilizer as a lever to disadvantage the growth and the seed production of the more nitrophilic weeds in favor of crop growth. Such a technique could help to develop cropping systems with reduced reliance on both nitrogen and herbicide inputs.

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