Biological nitrogen fixation of legumes crops under organic farming as driven by cropping management: A review

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1. Introduction

Agriculture is recognized as one of the main drivers of the global environmental changes and biodiversity loss (IPBES, 2016; IPCC, 2019). Organic agriculture is often proposed as an environmental and biodiversity-friendly way of farming (Bengtsson et al., 2005; Gattinger et al., 2012; Gomiero et al., 2011; Tuck et al., 2014). Yet organic crop yields are currently 20-25% lower compared to non-organically managed crops (De Ponti et al., 2012; Ponisio et al., 2015; Seufert et al., 2012). Thus, a deployment of organic farming, if not paired with an increase in crop yields, may lead both to insufficient agricultural production and to cropland expansion to match the food demand of growing world population. Such cropland expansion is likely to hinder most of organic farming's environment benefits (Meemken and Qaim, 2018; Mondelaers et al., 2009; Tuomisto et al., 2012). Therefore, sustaining crop productivity in organic farming is fundamental.

A number of empirical studies and meta-analyses have provided evidence that nitrogen (N) deficiency is a primary limiting factor of many crop species in organic systems (David et al., 2005; Ponisio et al., 2015; Seufert et al., 2012). According to some recent modelling exercises, organic crop productivity could be even more N-limited if organic farming was to drastically expand (Barbieri et al., 2021; Smith et al., 2018).

Several options exist to supply N in organic farming. The most promising one consists in increasing the share of atmospheric dinitrogen (N_2) fixing crops in organic crop rotations. Such increase may be achieved through various cropping practices. N fixing crops can be introduced in cropping systems as cash crops, cover and catch crops, temporary green manures, or in association with other crop species (i.e., intercropping, including as agroforestry). Providing estimates of the biological nitrogen fixation (BNF) of legume crops and how it varies with cropping options and crop species is of primary importance for designing productive organic cropping systems. At larger scale, it is also key to explore the safe "operating space" for feeding the world with organic agriculture under N constraints (Barbieri et al., 2021).

However, most papers (Cernay et al., 2016), reviews (Liu et al., 2011) and meta-analyses (Pelzer et al., 2014; Rodriguez et al., 2020; Yu et al., 2016) dealing with N-fixing crops have focused on their yield performance, while studies assessing their N_2 fixation ability are scarce (Anglade et al., 2015; Herridge et al., 2008; Peoples et al., 2021). In addition and surprisingly, very few studies have provided BNF estimates for organic farming systems and, to the best of our knowledge, no systematic estimates have been published regarding BNF of legume crops under organic management.

Several processes suggest that N_2 fixation is likely to differ in organically vs. conventional farming systems due to overall higher occurrence and/or susceptibility of organically managed crops to abiotic and biotic stresses. On the one hand, because organic farming generally experience low soil mineral N supply (Thomsen and Sørensen, 2006), the fraction of the plant N derived from the atmosphere relative to soil N uptake may be higher in organic systems (Elgharably and Benes, 2021). On the other hand, organic systems often experience higher biotic stresses due to weeds (Muneret et al., 2018), insects (e.g. the pea weevils (Corre-Hellou and Crozat, 2005)) and fungi (e.g. Aphanomyces), which may negatively impact their crop biomass productivity and/or increase its sensitivity to abiotic factor (such as drought). This results in generally lower symbiotic fixation in organic systems compared to conventional systems. As such, BNF estimates in organic systems cannot be inferred from the values measured in conventional systems.

In order to fill this knowledge gap, we provide here estimates of BNF in organically managed leguminous crop species, through the analysis of published data of direct measurement of BNF in such systems. Our analysis focuses on two key variables: the above ground N_2 fixation absolute values (Ndfa, in kgN. ha^{-1} yr^{-1}) and the share of the N in the crop biomass that is derived from the atmosphere through the BNF (%Ndfa), and their variations with crop species and cropping practices. We hypothesized that (i) Ndfa is higher in fodder crops compared to pulses due to differences in their biomass production; (ii) the lower availability of mineral N in organically managed soils compared to conventional farming systems increases %Ndfa but the higher biotic pressures reduces growth and the functioning of BNF itself, therefore presumably reducing Ndfa; (iii) the supply of organic fertiliser reduces Ndfa, since it may result in increased soil mineral N availability after the mineralization process of the organic inputs. We also expected that a high productivity combined with grain export of pulse crops species may result in negative net soil N budgets. Finally, we compare our results with values reported in the literature for non-organic farming systems.

2. Methods

2.1 Literature search and screening

We collected data on dinitrogen (N_2) fixation by organically managed leguminous crops through a systematic literature search in peer-reviewed journals and book chapters using the "*Web of Science*" platform. We used a complex Boolean search equation containing (i) the term *farm**, *system**, *agriculture or crop** near to (ii) the term *bean**, p*ea**, *lentil**, *soybean*, *lupin*, *vetch*, *alfalfa*, *clover*, *pulse** or *legume**, in combination with (iii) the term *nitrogen* or *N,* near to (iv) the term *recover**, *fix**, *acqui**, *contribit**, *redisu**, *suppl**, *uptake* or *benefit*. The last search was conducted on March 22nd, 2021, turning up 1008 publications.

The abstracts of these 1008 publications were screened to exclude (i) studies not focusing on legumes nor biological nitrogen fixation and (ii) studies not aligned with organic principles – based on the definition of organic agriculture given in the *Basic Standards for Organic Production and Processing* of the International Federation of Organic Agricultural Movement (IFOAM, 2014). This resulted in the selection of 504 publications which were further screened by selecting those that (i) were based on experimental data, (ii) explicitly stated that the experiments were carried out according to organic farming practices, and (iii) quantified N_2 fixation by legume crops based on empirical measurement. As such, we excluded, for example, studies that used %Ndfa estimates retrieved from literature for the calculation of Ndfa. The methods used for determining %Ndfa in the selected papers were (i) the difference method (Evans and Taylor, 1987), (ii) the dilution method (Chalk, 1985) and (iii) the natural abundance method (Shearer and Kohl, 1986). Following such criteria, the screening yielded in 39 publications reporting 538 observations on 76 distinct locations (sites) (***Figure S1 – Supplementary Dataset 1***). For each publication, an observation was defined as the combination of a given site, year, crop species, and set of agricultural practices (i.e. crop composition, sowing densities, fertilization rate). Only few publications reported comparative estimates of BNF for both organically and conventionally managed leguminous crops. Therefore, a direct comparative analysis between the two systems was not possible.

2.2 Data extraction

For each observation, we retrieved information on Ndfa [kgN. ha^{-1} yr^{-1}] (calculated either as above-ground N_2 fixation – for most publications – or as total plant N_2 fixation (including roots) – and referred here as $Ndfa_{total}$), the percentage of plant N derived from the atmosphere (%Ndfa) and the grain N yield (for pulses only). In addition, when available several characteristics of the study were included as moderators such as the legume crop species, the year and the geographic location (longitude and latitude), the legume crop cropping practices (see below), the soil type, whether the leguminous crop is organically fertilised or not, the sowing and harvesting dates, and whether the crop is irrigated or not (***Table 1***). We paid attention to the risk of double count when the results of the same experiment were reported in different publications. For a few articles, data were extracted from published figures using WebPlotDigitizer (version 4.5, https://automeris.io/WebPlotDigitizer/).

All observations were classified into (i) four crop types, namely pulses, fodders, pulse mixtures (defined as the mix of a grain pulse with a non-leguminous crop), and fodder mixtures (defined as the mix of a leguminous fodder with a non-leguminous fodder) and (ii) five cropping practices, namely pure cash crops, intercrops (defined as the simultaneous growing of two or more cash crop species in the same field), catch and cover crops (defined as crops that are grown between two main cash crops for no more than one year, thus not competing for land with cash crops), temporary leys (defined as fodder species that are grown instead of grain crop, for a maximum period of one year), and perennial crops (defined as fodder species grown for more than one year). For all mixtures and intercrops, we retrieved both the associated (i.e. non leguminous) crop species and the proportion of each species in the mixed crops, when available. The different crop species were defined using their scientific name, which was then related to their common name as referenced in the United States Department of Agriculture Plants Database (http://plants.usda.gov/java/). The sowing and harvesting dates were used to estimate the crop duration (in weeks). The Koeppen-Geiger climatic zone were determined for each experimental site based on their geographic coordinates using the R *kgc* package (Bryant et al., 2017).

Table 1. Main variables and moderators extracted from the articles.

Variable	Definition
Ndfa	Amount of N_2 fixed by the legume contained in the above-ground biomass [kgN. ha^{-1} yr^{-1}]. For mixed crops, Ndfa can either refer to the N_2 fixed by the legume on total field surface area bases (referred as $Ndfa_{measured}$), or only to the surface area sown within the legume crop within the intercrop.
$Ndfa_{total}$	Amount of N_2 fixed by the legume contained in the above-ground and below-ground biomass [kgN. ha^{-1} yr^{-1}].
%Ndfa	Percent of N in the legume above-ground biomass that is derived from N_2 fixation.
N_{grain}	Amount of N contained in the harvested grains [kgN. ha^{-1} yr^{-1}] (only for pulses).
N_{above_ground}	Total amount of N contained in the above ground biomass (i.e. atmosphere-derived and soil-derived N) [kgN. ha^{-1} yr^{-1}].
Crop type	Type of legume crop: pulse, fodder, pulse mixture, or fodder mixture.
Cropping practice	Type of legume cropping practice: cash crop, intercrop, catch and cover crop, temporary ley, or perennial.
Crop species	Legume species: chickpeas, vetches, velvet bean, soybean, lupine, common bean, jack bean, pea, fava bean, clovers, and alfalfa.
Mix composition	Proportion of each species in any mixed crop.
Year	Year of each observation.
Geographic location	Coordinates (longitude-latitude) of each site.
Soil type	Clayed (clay, silty clay and sandy clay), fine loamy (clay loam, sandy clay loam, silt loam and silty), coarse loamy (coarse loam, gravelly loam, loam, and sandy loam), sandy.
N fertilisation	Whether the crop is organically fertilised (N1: organic fertiliser applied) or not (N0: no fertiliser applied).
Sowing and harvesting dates	Sowing and harvesting of the crop [weeks 1-52].
Irrigation	Whether the crop is irrigated or not [yes-no].

2.3 Corrections for crop mixtures

Ndfa values in intercrops were first calculated on total field surface area basis, thus reflecting the Ndfa fixed by the total mixture on the total field area (hereinafter referred as $Ndfa_{measured}$). For the comparison of intercrops and mixtures BNF [in kgN. ha^{-1} yr^{-1}] with pure crops – either as pulses or fodders – we calculated Ndfa considering only the surface area sown within the legume crop within the intercrop.

For this, the corrected Ndfa, $Ndfa_{total}$, and N_{grain} values were re-calculated on a surface basis corresponding to a pure-crop plant density (Rodriguez et al., 2020) using Equation 1:

$$X_{mix} = \frac{X_{mix}^{I}}{\rho} \qquad \text{(Eq. 1)}$$

where X_{mix}^{I} is the legume Ndfa in the mixture, and ρ corresponds to the proportion of legume in the mixture relative to the sowing rate as pure crop. Note that %Ndfa data were not transformed for inter-crops and mixtures they do not vary with the surface area.

2.4 Additional calculations

Because Ndfa, %Ndfa and the total N in the above ground biomass are closely linked (Ndfa = total N × %Ndfa), when one of those three variables was missing, it was calculated based on the other two variables. In addition, when not available, $Ndfa_{total}$ was estimated by using crop-specific below-ground factors as reported by Anglade et al. (2015) (***Table S1***), thus accounting for the below-ground contributions from roots, nodules and rhizodepositions.

We also calculated a simple N budget for grain pulses when the N content in grains was reported, by using the following equation:

$$Pulse\ net\ N\ budget = Ndfa_{total} - N_{grain} = Ndfa * BG_{factor} - N_{grain}$$

Where BG factor refers to below-ground factors. This budget allows assessing whether pulses actually supply N to soils after harvesting (if the net N budget is positive) or if they contribute depleting soil N (if the net N budget is negative). Note that we assumed that all crop residues were returned to the soil when drawing this budget.

We computed the site effect by calculating the mean standard deviation of each legume species on each geographical site, as follows:

$$\overline{SD}_{site\ effect} = \frac{\sum SD_{crop\ species_{site}}}{n_{crop\ species_{site}}}$$

Where $\overline{SD}_{site\ effect}$ is the mean standard deviation (site effect), $SD_{crop\ species_{site}}$ is the standard deviation of each legume species in each geographical site and $n_{site_{crop\ species}}$ is the number of observations of each legume species of each geographical site.

2.5 Data quality checks and analysis

Data were screened for the presence of outliers, defined as any value below 1 kgN. ha^{-1} yr^{-1} or above 500 kgN. ha^{-1} yr^{-1} for Ndfa and below 1% or above 100% for %Ndfa. Outliers represented ~3% of all observations.

Since within-study variance measurements were available studies, individual observations were weighted by the number of replicates reported in the original studies (Pittelkow et al., 2015). All variables were analyzed using a bootstrap procedure (10,000 iterations, “ordinary” simulation type) to estimate their 95% confidence interval (CI) of each distribution (R *boot* package (Canty and Ripley, 2021)). The bootstrap procedure was performed on the weighted average of all observations within each variable and moderator. In order to limit bias effects, we only kept crop species that were present on at least two different sites (excluding ~11% of the observations). Since data did not always followed normal distributions, differences in Ndfa and %Ndfa among cropping practices and site conditions were tested using a non-parametric Kruskal-Wallis test.

3. Results

3.1 Above ground nitrogen fixation (Ndfa)

We found an overall 87 kgN. ha^{-1} yr^{-1} Ndfa value (95% CI: 75-99) when pooling all legume crop species and legume cropping practices (***Figure 1a***).

Considering legume crop "type", fodder crop species exhibited a significant higher mean Ndfa (131 kgN. ha^{-1} yr^{-1}) compared to grain pulses (76 kgN. ha^{-1} yr^{-1}), with alfalfa and clover showing the highest Ndfa (179 kgN. ha^{-1} yr^{-1} and 96 kgN. ha^{-1} yr^{-1}, respectively). Surprisingly, pulse and fodder in mixtures (i.e. when associated with a non-leguminous crop) had lower Ndfa values compared to cash crops. This suggests that pulse and fodder crop species may suffer from the competition for light, water or other nutrients with the associated non-leguminous crop, leading to lower biomass and resulting in lower Ndfa. Considering the effect of cropping practices, we found a significantly lower mean Ndfa for catch and cover crop crops (54 kgN. ha^{-1} yr^{-1}), compared to temporary leys (90 kgN. ha^{-1} yr^{-1}), and perennial legume crops (174 kgN. ha^{-1} yr^{-1}). Interestingly, intercrops did not result in overall higher Ndfa compared to cash crops (85 kgN. ha^{-1} yr^{-1} vs. 81 kgN. ha^{-1} yr^{-1}). These findings may result from differences in crop species, which strongly vary with legume cropping practices, with a six-fold difference between the highest and lowest Ndfa values (***Figure 1b***). Note also that we found higher Ndfa values for increasing crop growing period (i.e. perennial crops compared to catch and cover crops).

We also found large variability in Ndfa among legume species with alfalfa, clover and faba bean being the most performing species. Note that the large confidence intervals for Ndfa per crop (***Figure 1a)*** may reveal species specific response to geographic locations and pedo-climatic conditions, with some species showing a large variability (e.g. alfalfa), while others showing a narrower variability (e.g. vetch). Considering the relative contribution of the site and the species effects to the total variability (***Figure 2***), we found that the site effect (± 31 kgN. ha^{-1} yr^{-1}) accounted for approximately half of the total Ndfa variability (± 67 kgN. ha^{-1} yr^{-1}). This implies that the mean Ndfa values reported here should be used with awareness as Ndfa estimates on a specific geographical location.

We found a clear positive relationship between Ndfa and the crop above ground N biomass (***Figure 3)***. This confirms previous studies that showed that legume above ground N biomass is a good predictor of Ndfa (Anglade et al., 2015; Karpenstein-Machan and Stuelpnagel, 2000).

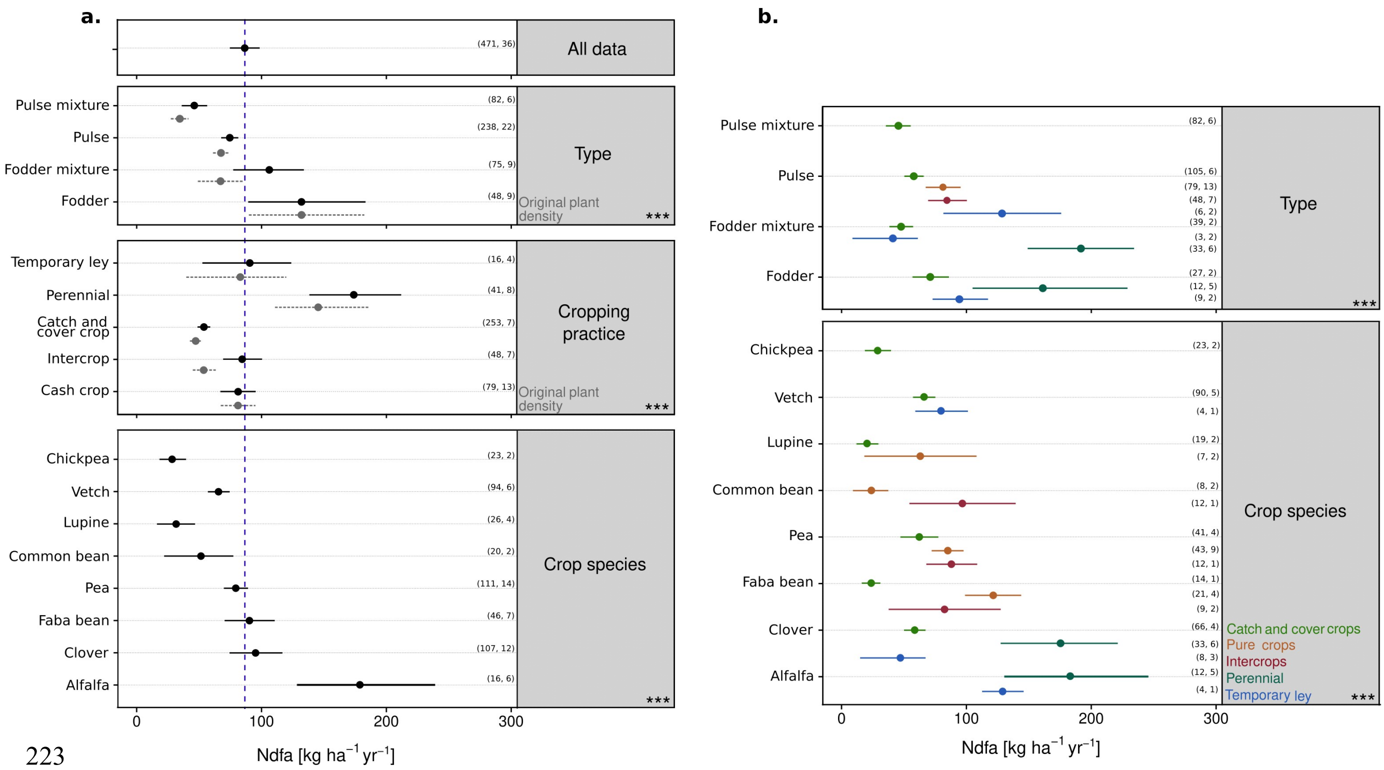

Figure 1. Above ground Ndfa means [kgN. ha^{-1} yr^{-1}] and 95% confidence intervals according to crop type, cropping practices, and crop species. a. Black symbols represent the Ndfa of legume mixtures when transformed into pure-crop plant density equivalent), and grey symbols represent the $Ndfa_{measured}$ by legume mixtures (see methods). b. Coloured lines indicate the cropping practices for crop type and crop species (using transformed values at pure crop equivalent density when in crop mixtures). The vertical dotted blue line represents the mean Ndfa when pooling all data. Numbers in parenthesis show numbers of observations and publications, respectively. ***: $p < 0.001$.

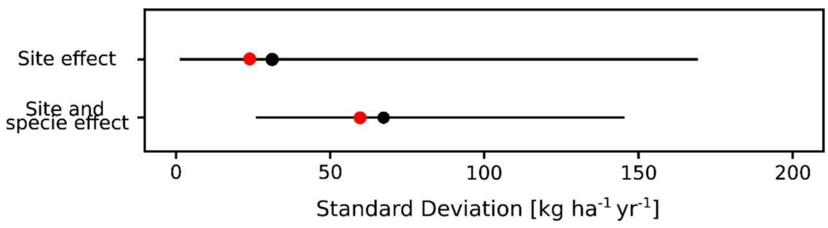

Figure 2. Standard deviation of legume species on each geographical site (thus representing the SD of the site – *site effect*) and standard deviation of all observations across all sites (*site and species effect*). Black points indicate the mean SD, red points indicate the median of the SD and lines indicate the range.

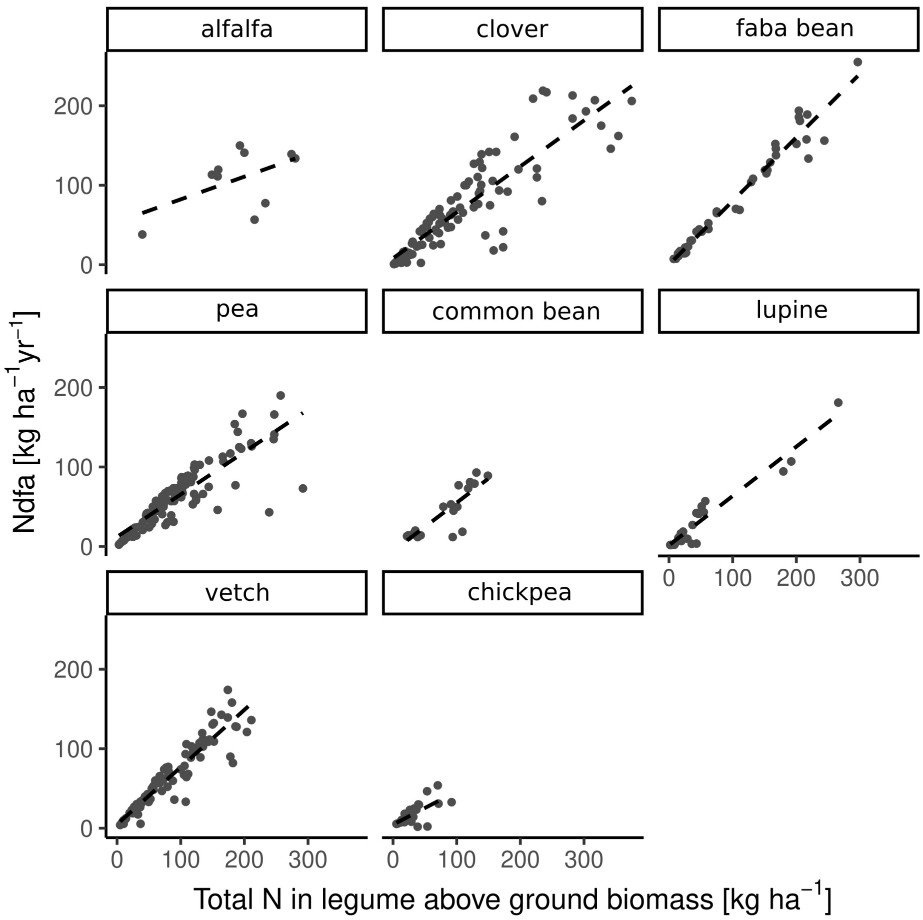

Figure 3. Relationship between the above ground N_2 fixation (Ndfa) and the N contained in the above ground biomass. Dotted lines represent fitted linear regression curves.

3.2 Percentage of N derived from the atmosphere (%Ndfa)

We found an overall mean %Ndfa of 69% (95% CI: 67-71), indicating that most of the N in legumes above ground biomass actually comes from the atmosphere (***Figure 4a***).

Considering the legume crop "type", as for Ndfa, fodders exhibited higher %Ndfa values (78%) than pulses (67%). We also found that pulses in mixture had higher %Ndfa than pure pulses (74% vs. 67%), thus suggesting that increased competition for resources leads to higher fractions of N retrieved from the atmosphere (Corre-Hellou et al., 2006). However, this result is not confirmed for fodders in mixture, which showed lower %Ndfa values than in cash crops (67% vs 78%; note that differences among crop "type" are significant at $p<0.05$ only between pure pulses and pulses in mixture).

Considering the effect of cropping practices, cash crops similarly exhibited overall lower %Ndfa values than all kinds of legume intercrops (58% vs. 74%, respectively) (***Figure 4a***). In addition, while higher Ndfa values were observed for increasing crop growing period (i.e. perennial crops compared to catch and cover crops, the average %Ndfa values of perennial crops (67%) was lower than for cover crops and catch crops (73%). But, the high variability of %Ndfa for perennial crop results of maximal %Ndfa values as high as for catch crops and cover crops (76%) but also with lower values (59%). High %Ndfa values for catch and cover crops may result from low soil mineral N availability in summer and autumn (low water availability in summer followed by fresh temperature in autumn) thereby stimulating their N_2 symbiotic fixation. In contrast, perennial crops benefit higher levels of N mineralization, as they grow over the whole year. Considering this relatively higher mineral N availability, favorable growth conditions for the perennial crop will lead to low %Ndfa while low growth will be associated with high % Ndfa values (Guinet et al., 2018).

Considering the crop species effect, we found a high variation in the %Ndfa among crop species ranging from 81% (faba beans) to 42% (common bean), with most values falling within a 65-81% range (***Figure 4a***). In addition, the %Ndfa of each crop species, varies strongly depending on the effect of different cropping practices (***Figure 4b***).

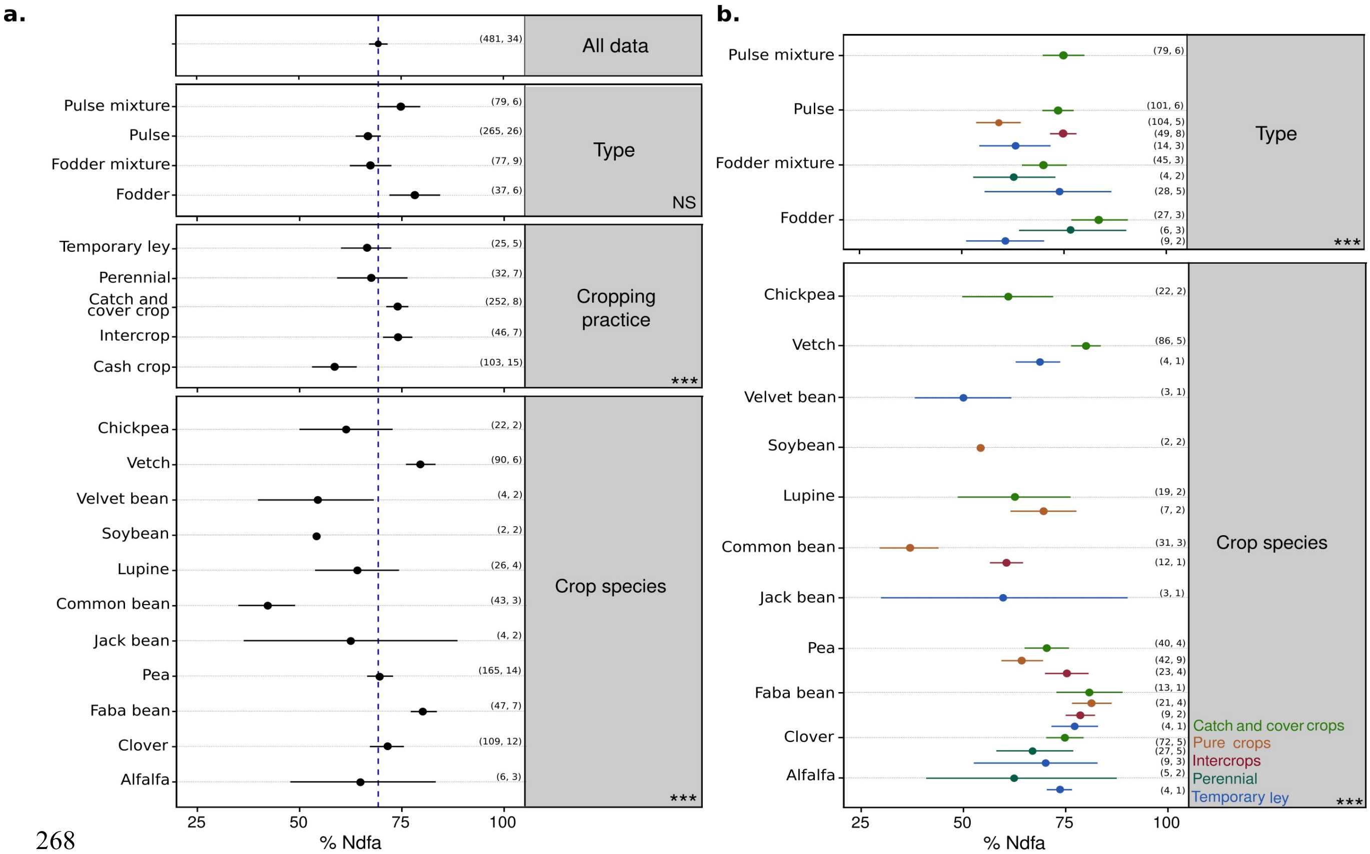

Figure 4. The percentage of N derived from the atmosphere (%Ndfa) means and 95% confidence intervals according to crop type, cropping practices, and crop species. Coloured symbols (panel b) indicate cropping practices for each crop type and crop species. The vertical dotted blue line represents the mean %Ndfa when pooling all data. Numbers in parenthesis show numbers of observations and publications, respectively. ***: $p < 0.001$; NS: non-significant.

3.4 N_2 fixation as a function of crop duration

One major difference between different cropping practices and crop types is the average length of the cropping period. We found a relationship between the duration of the cropping period and the weekly $Ndfa_{measured}$ (***Figure 5***). Given the relationship between the $Ndfa_{measured}$ and the total N in above ground biomass (***Figure 3***), the weekly $Ndfa_{measured}$ rate can be explained by the crops growing rate. Therefore, the darker points in ***Figure 5*** indicate crop species (***panel b***) and pedo-climatic conditions (***panel a***) characterized by high and fast N_2 fixation rates. On one hand, short cropping duration (10 to 20 weeks from sowing to harvest) are mostly correlated with high weekly fixation rates, although sub-optimal growing environment can sometimes result in lower weekly fixation rates (lighter points). On the other hand, longer cropping duration are associated with small fixation rates (below 25 $kgN.ha^{-1}$), and, thus, with crops species characterized by a slower growing rate. This suggests that there might be a trade-off between N fixation rate and N fixation duration. This relationship between the $Ndfa_{measured}$ and the duration of the cropping period is also confirmed by ***Figure S4*** which shows a weak but positive correlation ($R^2 = 0.32$) between the number of weeks from sowing to harvesting and the $Ndfa_{measured}$, for different cropping practices.

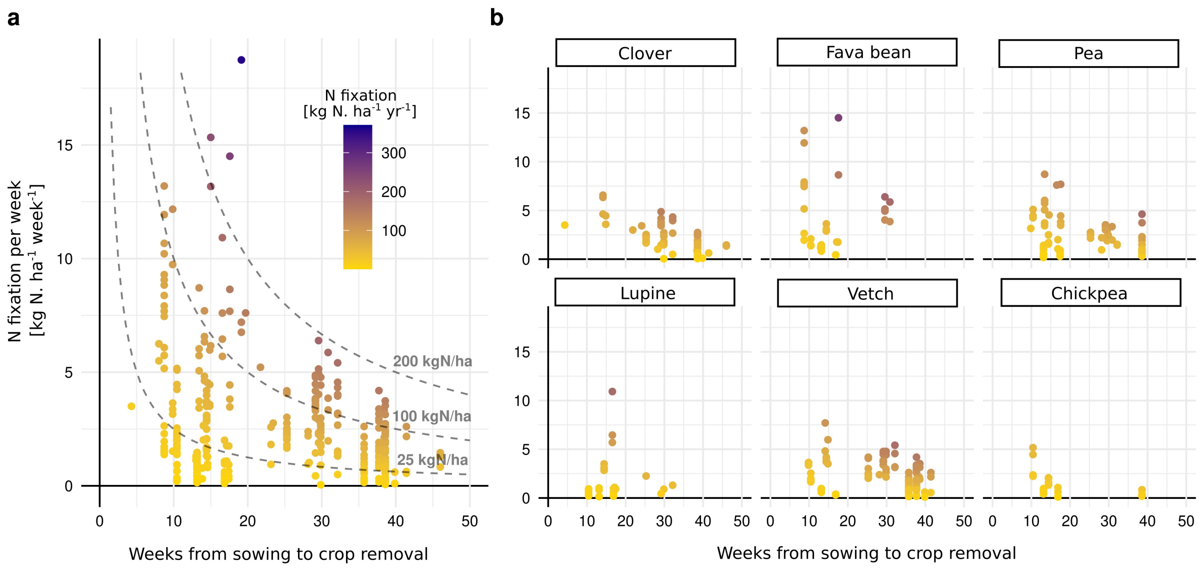

Figure 5. Above ground N fixation ($Ndfa_{measured}$) per week as a function of time from sowing to harvest, (a) : for the overall data set and (b) : for a selection of crop species We calculated the mean above ground N fixation per week by dividing above ground N fixation by the duration from seeding to harvest, expressed in weeks. Color scale indicates the level of $Ndfa_{measured}$, as measured from sowing to harvesting. Dotted lines indicate the iso-N fixation values from sowing to harvesting.

3.5 Net pulse N budget following grain harvest

Net soil N budgets for grain pulses (see Methods) indicate whether the grain harvest offsets N input through N_2 fixation. We found that in 20% of the observations, the net N budget was lower than −10 kg N. ha^{-1}, 33% of cases lead to a balance in between −10 and +10 kg N. ha^{-1}, and 47% of cases lead to a balance superior to +10 kg N. ha^{-1}. Thus, we found negative net soil N budgets for one third out of the 40 observations that reported the amount of N contained in the harvested grains (***Figure 6***). In those situations, pulses cultivation and their management practices contribute to soil N depletion. We also found a general negative correlation between the N amount in the harvested grain and the net soil N budget (***Figure 6***) which underlines a trade-off between the production of high quantities of edible protein (for feed or food) and the supply of N to soils through biological N fixation. Note that most available data for this analysis referred to pea. Note also that the lower the net N budget, the lower the %Ndfa values, showing that negative N budgets often occur when soil mineral is high.

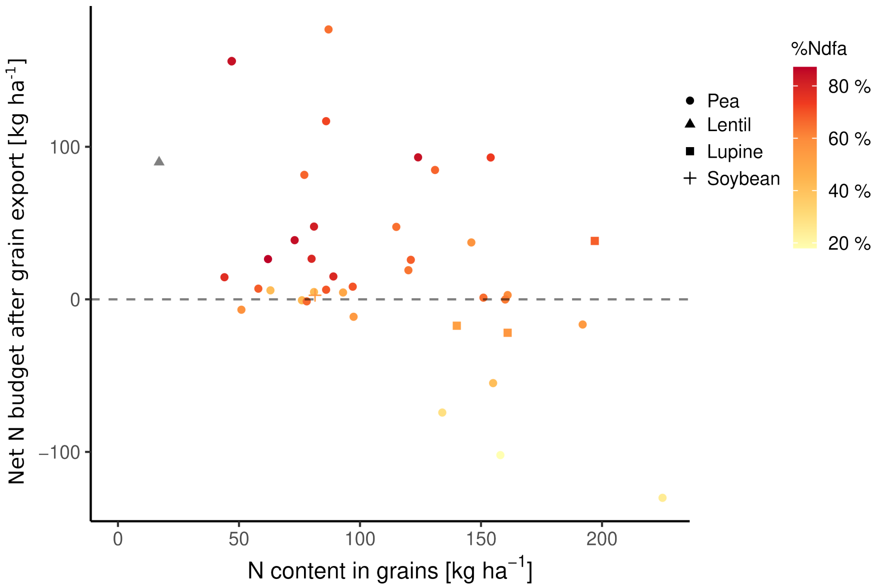

Figure 6. Relationship between pulse N budget and N contained in the harvested grains. The N balance is calculated as the difference between total N coming from the atmosphere ($Ndfa_{total}$) and the amount of N in the harvested grains. Positive balances represents situations where pulse cultivation actually provides N to soils. Symbols represent crop species and yellow to red colours indicate the corresponding %Ndfa value.

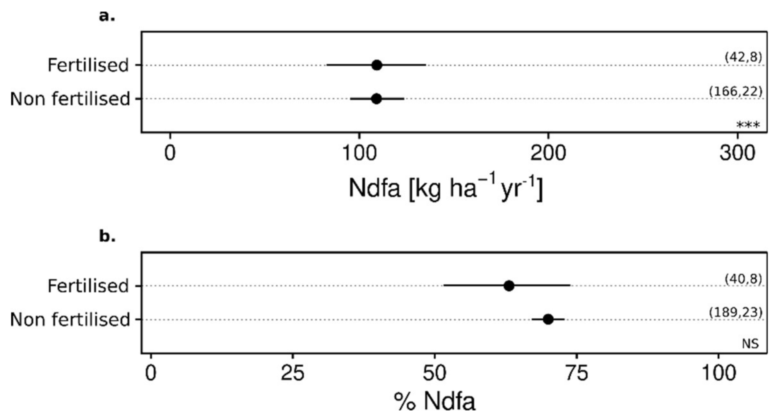

Figure 7. Means and 95% confidence intervals of BNF as affected by organic N fertiliser application (**a.**) Above ground N_2 fixation (Ndfa) and (**b.**) percentage of N derived from the atmosphere (% Ndfa). Numbers in parenthesis show number of observations and publications, respectively. ***: $p < 0.001$; NS: non-significant

3.6 Comparison with non-organic farming systems

We compared our results - representative of organically managed farming systems - to Ndfa and %Ndfa values reported in the literature (Cernay et al., 2016; Herridge et al., 2008; Kakraliya et al., 2018; Peoples et al., 2021; Smil, 2001) for non-organic – i.e. conventional – farming systems (since conventional agriculture represents most of the cultivated area, and since the authors did not mention organic farming). Although showing variations according to crop species, mean Ndfa values in organic farming (this study) were often lower than those reported in the literature (***Table 3***). These lower Ndfa values may be related to the overall lower yield of organic vs. conventional crops and, thus, lower biomass production (leading to a lower N demand) (De Ponti et al., 2012; Ponisio et al., 2015; Seufert et al., 2012). In addition, a higher pressure of abiotic stresses (i.e. water stress and soil compaction) in organic compared to conventional farming can also directly impact the N_2 fixation by affecting the roots nodules (Santachiara et al., 2019).

In contrast, we found that %Ndfa values in organic farming were generally similar to their conventionally counterpart (excluding clover, for which we found a lower %Ndfa in organic farming systems) (***Table 4***). Given the overall lower availability of mineral N in organic systems (Lampkin, 1990), we would have expected higher fractions of biomass N coming from the atmosphere. Therefore, based on the reported data, we could not confirm our hypothesis of higher %Ndfa values in organic vs. conventional farming systems. Actually, both soil mineral N availability and crop growth potential (driving N demand for BNF) were lower in organic farming, resulting in similar % Ndfa in organic and conventional systems.

Table 3. Comparison of Ndfa [kgN. ha^{-1} yr^{-1}] under organic management (this study) with values reported in the literature for conventional farming systems. Values between brackets represent either the 95% confidence interval or the range. Note that values in italic were calculated as the unweighted average of the values reported in this table.

	This study	Smil et al. (2001)	Anglade (2015)	Herridge et al. (2008)	Kakraliya et al. (2019)	Peoples et al. (2021)	Average difference
Common bean (Phaseulus vulgaris)	52 (22-78)	40 (30-50)		16	50 (20-80)	75	*+7 (+15%)*
Pea (Pisum sativum)	80 (70-89)	40 (30-50)	82 (42-121)	61	83 (65-100)	105	*+6(+8%)*
Chickpea (Cicer arietinum)	29 (19-39)				45 (40-50)	48	*-18 (-37%)*
Fava bean (Vicia faba)	90 (71-111)	100 (80-120)	139 (88-167)	76	130	135	*-16 (-22%)*
Lupine (Lupinus L.)	32 (17-47)				80 (60-100)		*-48 (-60%)*
Soybeans (Glicine max)		80 (60-100)		117	125 (100-150)	144	*NS*
Clover (Trifolium L.)	96 (75-116)	150 (130-170)	77 (38-158)		125 (100-150)		*-21 (-18%)*
Alfalfa (Medicago sativa)	179 (129-240)	200 (150-250)	70 (40-121)				*+44 (+33%)*
Pulses	76 (68-83)	60 (40-80)	*64*	*60*	*85*		*+8 (+10%)*
Fodders	131 (90-182)	100 (80-120)	*74*	85	*125*		*+35 (+36%)*
All legumes	87 (75-98)	*96*	*92*	*69*	*94*	101	*-3 (-4%)*

Table 4. Comparison of the %Ndfa under organic management (this study) with values reported in the literature for conventional farming. Values between brackets represent either the 95% confidence interval or the range. Note that values in italic were calculated as the unweighted average of the values reported in this table.

Moderator	This study	Cernay et al. (2016)	Peoples et al. (2021)	Average difference
	%Ndfa	*%Ndfa*	*%Ndfa*	*%Ndfa*
Common bean (Phaseulus vulgaris)	42 (35-49)		37 (27-47)	*+5 (+14%)*
Pea (Pisum sativum)	69 (66-73)	67 (20-85)	65 (54-76)	*+3 (+1%)*
Chickpea (Cicer arietinum)	62 (50-73)	62 (1-88)	61 (51-73)	*+0.5*
Fava bean (Vicia faba)	80 (77-83)	82 (26-99)	74 (63-85)	*+2 (+2%)*
Lupine (Lupinus L.)	64 (53-74)	69 (26-87)	70 (58-82)	*-5.5 (-8%)*
Soybeans (Glicine max)	54	60 (43-79)	59 ± 18	*-5.5 (-9%)*
Clover (Trifolium L.)	72 (68-76)	89		*-11 (-19%)*
Alfalfa (Medicago sativa)	65 (48-83)			
Pulses	78 (72-75)	*68*	62 ± 18	*+13 (+20%)*
Fodders	67 (64-70)	*89*	/	*-22 (-24%)*
All legumes	69 (67-71)	*72*	60 ± 17	*+3 (+5%)*

4. Discussion

Our study proposes novel estimates for Ndfa and %Ndfa under organic farming management. This specific information is of great importance since N_2 fixation by leguminous crops is often given key role in organic systems.

Our results show that N_2 fixation in organic farming is strongly correlated to the above ground N biomass, in agreement with previous literature in conventional systems (Guinet et al., 2018). Indeed, in both types of agricultural systems, Ndfa is mainly driven by plant growth and the associated N needs. This suggests that the maximisation of symbiotic N inputs by leguminous crops at the field scale requires maximising legume biomass production. To this purpose, optimising crop managements by providing good control of biotic and abiotic stresses is of primary importance. Any stress that impacts the legume plant growth may have indirect consequences on legume crop N demand and therefore on its potential to accumulate atmospheric nitrogen. Especially biotic stress such as weed competitions, insects and diseases, which are common in organic farming (Stockdale et al., 2006), may lead to lower biomass production and, thus, to lower Ndfa. By their action on roots, some of these stresses may also lead to increase legume sensitivity to water stress, and therefore further reduce plant growth. Besides, a number of biotic and abiotic stresses directly impact symbiotic nodule integrity (sitones, aphanomyces) and/or their ability to fix N_2 (drought, lack of oxygen due to soil compaction or water lodging) (Doré et al., 1997).

Due to their higher above and below ground biomass production, fodders species exhibited higher Ndfa and $Ndfa_{total}$ in comparison to pulses in organic farming. This higher biomass production may be due to their longer cultivation duration compared to pulses (***Figures 5 and S3***). As such, introducing fodder crops in organic crop rotations is an important strategy for managing N fertilisation (Barbieri et al., 2017). Nevertheless, it must be noted that in the fields most fodder leys associate legumes and non-legumes crop species (fodder mixtures, ***Figure 1***), thus reducing Ndfa per hectare in comparison to cash crops (***Figure 1a –*** $Ndfa_{measured}$).

It is unclear how organic N fertilisation can impact N fixation in organic farming systems. ***Figure 7*** shows a decrease in the %Ndfa associated with stable Ndfa absolute values when organic fertiliser are applied. This suggests that, in such cases, the soil mineral N supply stands-in for the reduction in the %Ndfa. Thus, our analysis showed that the effect of organic fertilisers on Ndfa and %Ndfa may be similar to an increase of mineral N, despite the temporal delay between the organic fertiliser application and the mineralisation processes (Watson et al., 2002). Such results also confirms that the biomass production of organic leguminous can be limited by a lower availability of soil mineral N as compared to conventionally managed crops.

***Figure 6 and** 7* also suggest that a high availability of soil mineral N negatively impacts the soil N budget of grain pulses. In such cases, the soil N inputs through crop residues incorporation following grain harvest is lower than N input through BNF resulting in a negative N net budget. As a result, including pulses in organic crop rotation with high level of organic fertiliser may not supply N to the cropping system and should thus be complemented with other management strategies for providing N.

In organic agriculture, there is generally a trade-off between restoring soil fertility, e.g. by using green manure and leguminous fodder leys, and enhancing food production (Connor, 2018). Indeed, green manures and fodder leys occupy fields without producing direct food for human consumption – even if they may be used as animal feed. In order to attenuate trade-off, organic farmers may minimise the land competition by non-cash crop legumes during the rotation. This could be done by, for instance, cultivating legume cover crops or green manure outside the growing season of cash crops. In this perspective, crop species assuring high Ndfa over short period of time should be favoured (***Figure 5***).

The Ndfa and %Ndfa estimates provided here are particularly relevant for organic farming perspective scenarios since they strongly rely on legume fixation as an N input. To this purpose, we compared our $Ndfa_{total}$ and %Ndfa estimates with the ones used in studies simulating organic farming expansion at the global, European and national scales (***Table S2***) (Barbieri et al., 2021; Billen et al., 2021; Poux and Aubert, 2018; Smith et al., 2018). We did not find significant differences in the $Ndfa_{total}$ except for specific crop species or studies. For example: for lupine and alfalfa we found lower and higher $Ndfa_{total}$ values than the ones used in the literature, respectively. We also found that the values used for fodders often differed compared to our results, i.e. as in the Ten Years For Agroecology (TYFA) foresight (Poux and Aubert (2018)). Overall, when comparing studies by aggregating all crop species, the parameterisation of organic scenarios is generally in line with the values reported in this study (average difference <10%).

Another feature emerging from our analysis is the high variability of Ndfa values. ***Figure 2*** shows that even in the same site, Ndfa standard deviation can be relatively high compared to the total variation. This might do to the stronger influence in organic vs. conventional farming of both inter-annual climatic variation and higher sensitivity to pest and diseases on crop productivity. Two meta-analyses found that organic agriculture experiences higher yield variability than conventional agriculture (Knapp and van der Heijden, 2018; Smith et al., 2019). Thus, the mean Ndfa values reported here should be carefully used as estimates for Ndfa values on a specific geographical location. Note also that Ndfa variability is higher the longer the duration of the cropping period: perennial and temporary leys show higher confidence intervals than all other management strategies. Therefore, despite perennial crops showed the highest Ndfa values, their performance may was also highly variable due to the higher probability of incurring in sub-optimal growing conditions.

In addition, this observed variability may challenge the use of mean Ndfa values when parameterising N-fixation in model-based studies notwithstanding of the geographical region. Our analysis can help introducing some quantitative variability in such modelling exercises.

Our study comes with a few limitations. Similarly to previous reviews and meta-analyses (Barbieri et al., 2017; Ponisio et al., 2015; Seufert et al., 2012), our dataset is characterized by a bias towards developed countries and especially Western Europe (***Figure S2***). Note that several publications reporting data from Africa and South America were excluded since they did not report experimental measurement of N_2 fixation – which was one of the exclusion criteria we adopted during the literature screening. With respect to the representativeness of the different crops, some species were underrepresented in our dataset. Above all, only two publications reported data on soybean, despite being the most cultivated leguminous crops in the world (Food and Agriculture Organization of the United Nations, 2022). This may be explained by the fact that (i) most of our publications come from Europe, a region were soybean is rarely grown (Food and Agriculture Organization of the United Nations, 2022) and (ii) soybean is less frequent in organic compared to conventional farming (only 0.6% of the global soybean area was grown organically vs. ~1.5% of global croplands (Agence Bio, 2020)). Likewise, some grain legumes for human consumption – e.g. lentils – were not represented in our dataset, while we could collect data for more minor crop species – e.g. velvet bean. This suggests that the experimental literature about organic legumes focuses more on innovative systems and may be less representative of current organic farmers' practices.

5. Conclusion

Our quantitative review provides useful information for both quantifying and understanding biological nitrogen fixation under organic management at the cropping system level. Our estimates can help improving model-based scenarios projecting an expansion of organic farming at large spatial scales. Overall, we found a large variability in legume N_2 fixation rates, even within one single species. Our analyses also showed that pulses may have, in contexts of high soil N availability, null or even negative net contribution to soil N budgets once grains are exported. This highlights the importance of introducing non-food crops in organic rotations to increase soil N supply. However, because non-food crops may be used as green manure, they may contribute to decreasing the food productivity of organic cropping systems. Taking advantage of the variability observed among crop species and cropping practices may be a way to overcome this issue.

Author statement

P.B, T.S., and T.N. jointly conceived the study. T.S. run the literature search, T.S. and P.B run the statistical analysis. P.B., T.S., A.S.V., and T.N. contributed writing and editing the paper.

Declaration of Competing Interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary information

Biological nitrogen fixation of legumes crops under organic farming as driven by cropping management: a review.

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Supplementary Figures

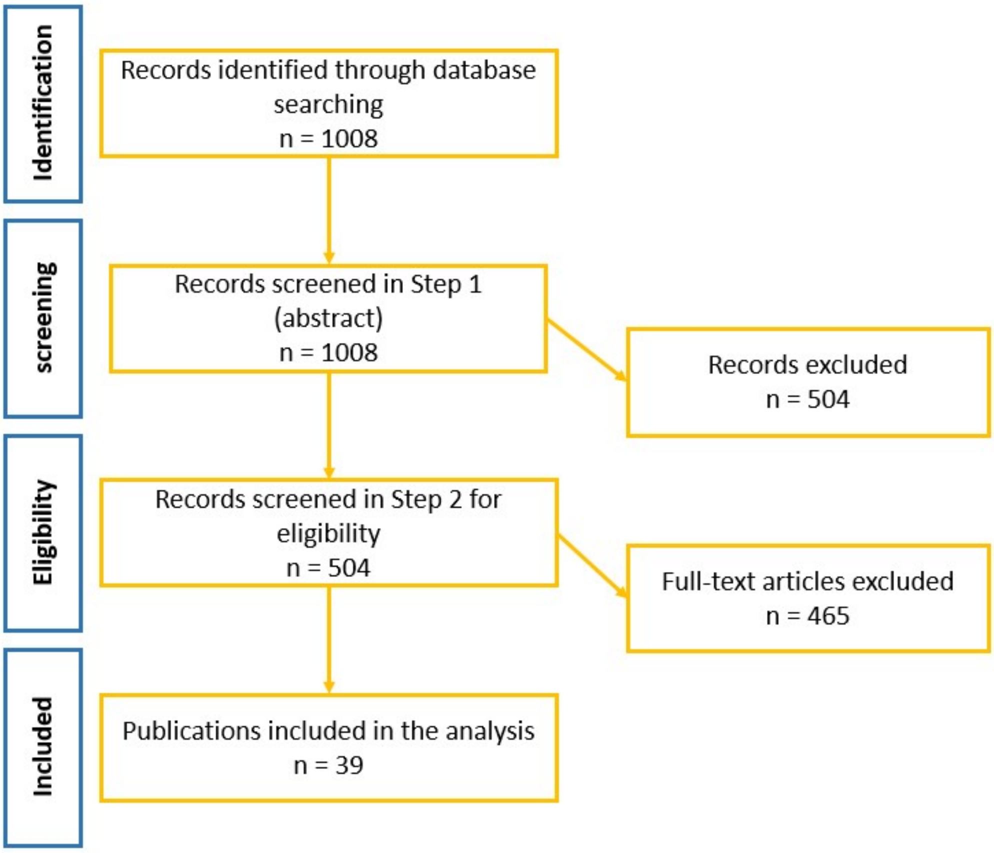

Figure S1. PRISMA flow diagram.

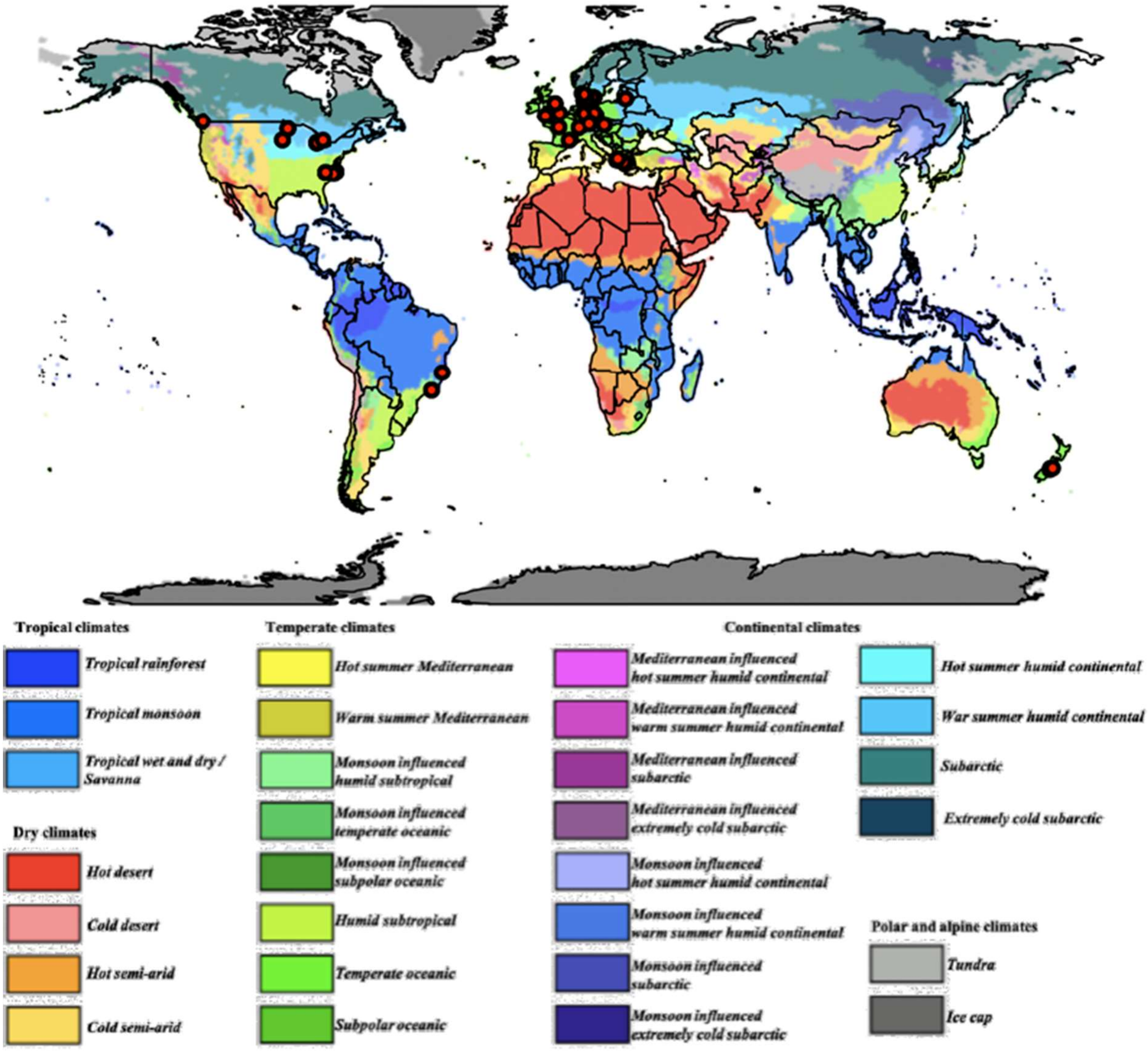

Figure S2. Geographical distribution of the 75 experimental sites present in the review (red points), and their climatic zone according to the Köppen-Geiger climate classification system.

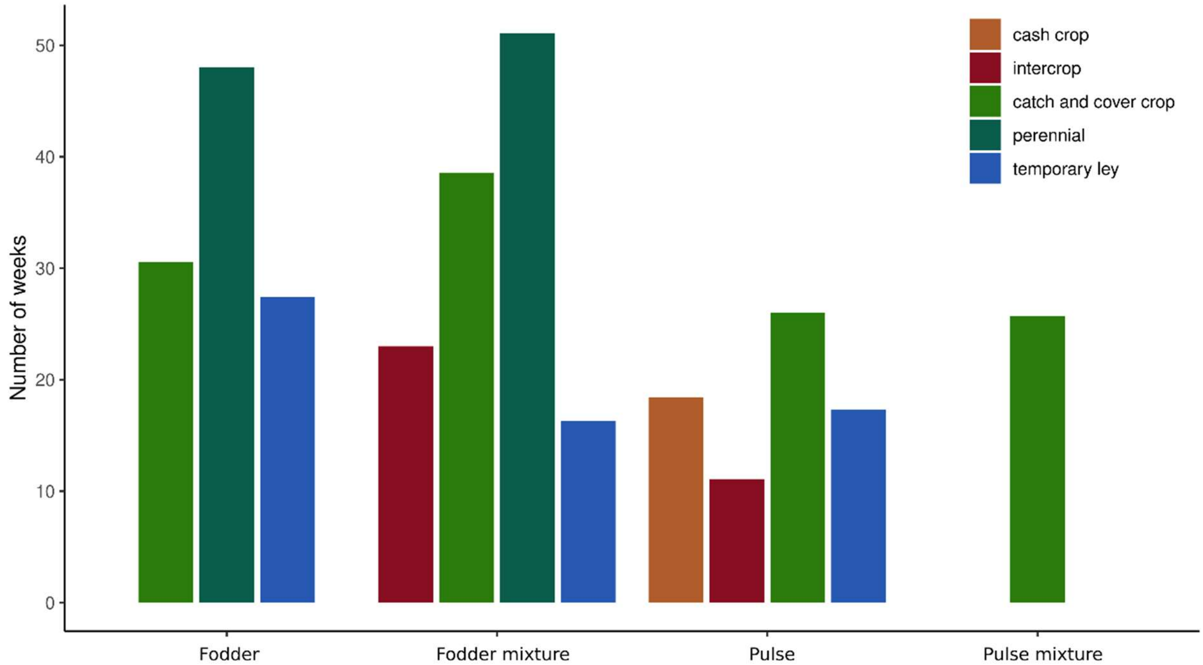

Figure S3. Mean number of weeks from seeding to harvest for each crop type and crop management strategies.

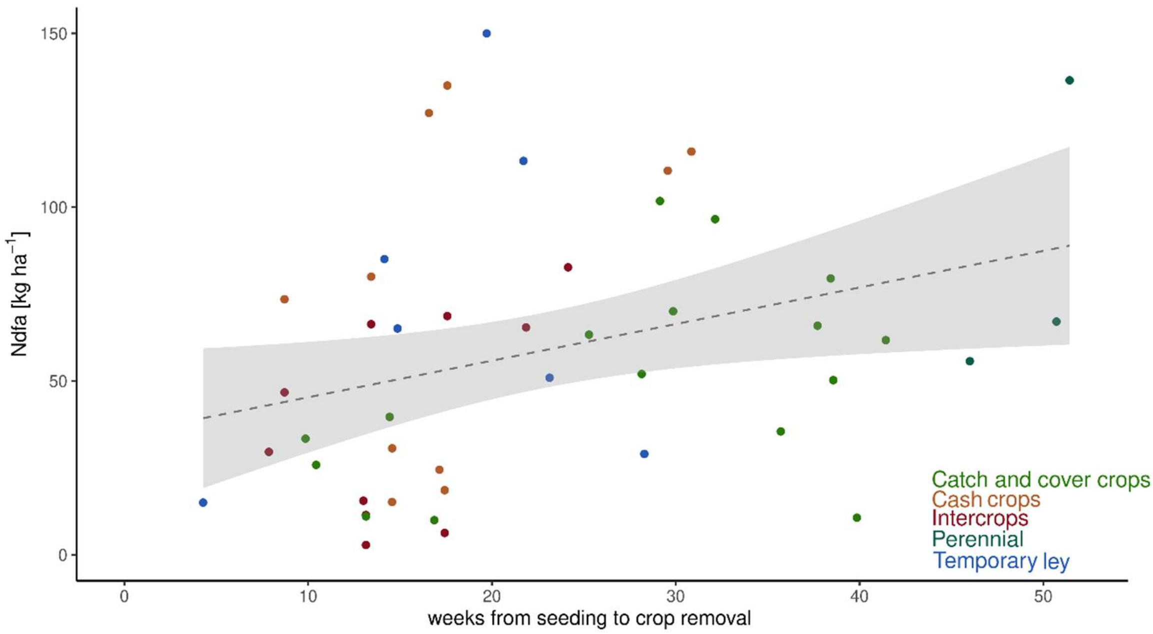

Figure S4. Above ground N_2 fixation (Ndfa) as a function of time from sowing to harvest. Colours indicate the different management practices. The dotted line represent a regression curve with 95% confidence intervals (grey shade). Note that the correlation is weak ($R^2 = 0.32$). *: $p < 0.05$.

Supplementary tables

Table S1. *Below-ground factors for all crop species considered in this study, from Anglade et al. (2015)*

Crop species	Below-ground factor
Alfalfa	1.7
Clover	1.7
Lentil	1.3
Fava bean	1.4
Field pea	1.3
Fodder (generic)	1.7
Grain pulses (generic)	1.3

Table S2. Comparison of $Ndfa_{total}$ [kgN. ha^{-1} yr^{-1}] under organic management (this study) with values used as parameters to estimate nitrogen fixation in organic upscaling scenarios in Europe. Values between brackets represent either the 95% Confidence Interval, the range or the Standard Deviation. Note that the values in italic – including the average difference – were calculated as the unweighted average of the values reported in this table.

Moderator	This study	Billen et al. (2021)	Poux and Aubert (2018)	Barbieri et al. (2021)	Smith et al. (2018)	Average difference
Common bean (Phaseulus vulgaris)	67 (28-100)	56		64 ± 18		*+7 (+12%)*
Pea (Pisum sativum)	103 (91-116)	105 ± 54		86 ± 23	130 (28-215)	*-4 (-3%)*
Chickpeas (Lathirus sativus)	38 (24-51)	50		42 ± 17		*-8 (-17%)*
Fava bean (Vicia faba)	126 (99-155)			95 ± 25	138 (73-211)	*+10 (+10%)*
Lupine (Lupinus L.)	42 (22-61)				190 (110-270)	*-148 (-78%)*
Vetch (Vicia L.)	114 (99-129)	103		115 ± 101	170 (100-240)	*+5 (+4%)*
Clover (Trifolium L.)	162 (127-197)	265 (185-285)		165 ± 77	244 (134-354)	*-62 (-30%)*
Alfalfa (Medicago sativa)	304 (219-408)	181 (149-260)		210 ± 34		*+109 (+55%)*
Pulses	87 (79-95)	94 (61-154)	58 (43-68)	89 ± 23		*+7 (+9%)*
Fodder	224 (152-310)		340 (300-380)	131 ± 85	170 (110-227)	*+10 (+4%)*
Fodder mixture	115 (83-145)	72 (63-87)	122	62 ± 43	215 (70-360)	*-3 (-2%)*
Total average	129 (112-148)	*118*	*173*	*81*	*179*	*-9 (-6%)*

Table S3. List of the crops species reported in the original publication that have been merged into common crop species categories for the analysis of this study.

Crop species retained in this study	Crop species reported in the original publications
Clover	Red clover, white clover, alsike clover, crimson clover, subterranean clover, reversed clover, Egyptian clover
Vetch	Winter vetch, garden vetch
Lupine	Narrowleaf lupine, European yellow lupine