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# Grain legume-cereal intercropping enhances the use of soil-derived and biologically fixed nitrogen in temperate agroecosystems. A meta-analysis

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ABSTRACT

Grain legumes are known for their benefits to deliver ecosystem services on provisioning of protein-rich food and feed, reducing greenhouse gas emissions through the symbiotic nitrogen fixation function and diversification of cropping systems. Intercropping is an agroecological practice in which two or more crop species are grown simultaneously in the same field, thereby maximizing the use of resources to enhance yields in low input systems and the resilience of cropping systems. We quantified the effect of grain legume-cereal intercropping on the use of N resources in temperate agroecosystems, focusing on dinitrogen ($N_2$) fixation and soil-derived nitrogen acquisition using a meta-analysis of 29 field-scale studies. We estimated and compared effects of different intercrop compositions (proportion of each species in the intercrops), fertilization rates, crop species, soil properties, and other management practices on the symbiotic $N_2$ fixation and the acquisition of soil-derived nitrogen by the cereals and grain legumes. The proportion of N derived from $N_2$ fixation was on average 14 % (95 % CI = [11, 16]) higher in intercropped grain legumes (76 %) compared to legume sole crops (66 %). On the other hand, intercropping reduced the amount of $N_2$ fixed (kg $ha^{-1}$) by about 15 %, when $N_2$ fixation in inter- and sole cropped legumes was expressed at equivalent density by compensating for the sown legume proportion in intercrops relative to their sole crop sowing rate. The results were mainly influenced by the intercrop composition, legumes species and the method used to quantify $N_2$ fixation. Soil-derived nitrogen acquisition in intercropped grain legumes was significantly reduced (−47 %, 95 % CI = [−56, −36]) compared to sole crop legumes, expressed at equivalent density, while the soil N acquired by intercropped cereals was much higher (+61 %, 95 % CI = [24, 108]) than in sole crop cereals. Total soil N acquisition (legume + cereal) was significantly higher in intercrops than in legume sole crops (+25 %, 95 % CI = [1, 54]), while there was no significant difference between intercrops and cereal sole crops. The meta-analysis confirms and highlights that intercropping consistently stimulates complementary N use between legumes and cereals by increasing $N_2$ fixation by grain legumes and increasing soil N acquisition in cereals. Based on the results of this analysis it would be suggested that cropping systems diversification via intercropping can be used for simultaneous production of both cereals and grain legumes, while increasing the use of N-sources and reducing external inputs of N fertilizers, thereby enhancing the sustainability of agriculture.

## 1. Introduction

The intensification of cereal-based cropping systems has negatively impacted the environment (Altieri, 1998; FAO, 2008), resulting in eutrophication of ecosystems, increased greenhouse gas emissions, and loss of biodiversity (Millennium Ecosystem Assessment, 2005). Agroecology and ecological intensification haven been promoted as approaches to reduce these impacts, thus increasing the interest in diversified cropping systems in the research agenda. Diversification of cropping systems is suggested as a positive strategy to support functional biodiversity (FB) at field, farm and landscape levels (Kremen et al., 2012; Bommarco et al., 2013). The FB concept encompasses plant genetic and plant varietal diversity, intercropping, pastures, hedgerows, agroforestry, cover crops, crop rotation, fallow periods, riparian buffers, etc. (Shennan, 2008; Kremen and Miles, 2012). Increased on-farm diversity enhances the capacity of agroecosystems to maintain soil

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fertility, regulates water use, reduces the pressure of pests and diseases, and upholds better yield (Scherr and McNeely, 2008; Malézieux et al., 2009). Management of cropping systems using several approaches such as intercropping, cover crops and crop rotations may therefore be important strategies to promote FB by increasing species richness (Connolly et al., 2001) and by enhancing the functioning of ecosystems processes via altered or improved resource acquisition, partitioning and niche differentiation (Moonen and Bàrberi, 2008).

Legumes have traditionally been used in cropping systems, as part of crop rotations and also intercropped with other crops (especially cereals). The key benefits of legumes include the biological fixation of atmospheric nitrogen through a symbiotic relationship with soil bacteria collectively referred to as rhizobia (Hauggaard-Nielsen et al., 2003; Peoples et al., 2009; Jensen et al., 2010). Furthermore, legumes play an important role in reducing greenhouse gas emission (Jensen et al., 2012; Jeuffroy et al., 2013), due not only to their capability for dinitrogen ($N_2$) fixation that would replace fertilizers based on fossil energy, but also for carbon sequestration and biomass production (Jensen et al., 2012). Moreover, legumes provide multiple ecosystem services such as biological control, pollination, carbon sequestration and nutrient cycling (Döring et al., 2012; Blanco-Canqui et al., 2015; Gaba et al., 2015). Indeed, legumes have great potential to diversify farming systems to restore associated biodiversity in agroecosystems, and serve as break-crops which help to reduce weeds, pest and disease associated with short rotations (Voisin et al., 2013). Grain legume crops may also increase yields of subsequent cereals by about 29 % on average (Cernay et al., 2018). Finally, legumes are a sustainable source of protein for healthy human and animal diets (Voisin et al., 2013). However, despite their multiple benefits, grain legumes remain minor crops in European agriculture, where their cultivation has been partly displaced by the expansion of cereal and oilseed crops during the last fifty years (Murphy-Bokern et al., 2017). Certainly, the production of grain legumes faces diverse problems, which include the yield variability and the difficulties to control pests and weeds (Magrini et al., 2016; Zander et al., 2016; Watson et al., 2017). But, having legumes intercropped with non-legumes (i.e. cereals) could be a way to favour yield stability (Raseduzzaman and Jensen, 2017) and weed control (Corre-Hellou et al., 2011; Bedoussac et al., 2015).

Intercropping is an agroecological practice, where two or more species are growing simultaneously in the same field, at least a part of the growing season (Willey, 1979). Plant interactions in intercrops may take various forms a) competition, b) complementarity, c) facilitation and d) compensation (Tilman, 1999; Cardinale et al., 2002). The advantages of intercropping in farming systems are often associated with more efficient use of resources (Vandermeer, 1989; Francis, 1990). In fact, the increase in total yield in intercrop may be due to the differences in growth factor requirements of the intercrop component crops (Gliessman, 1985), or to the complementarity in use of N-sources (Hauggaard-Nielsen et al., 2008), or to the facilitation of interplant N transfer and availability of phosphorus (Jensen, 1996a; Hinsinger, 2001; Li et al., 2009b), and to yield stability by the compensation effect (Rao and Willey, 1980; Tilman, 1996).

Meta-analysis is now frequently used to combine a large number studies to assess how one or several variables respond to experimental treatments that are common across studies. Such quantitative syntheses provide analytical techniques to estimate the effects of cropping practices and to analyse their uncertainties (Philibert et al., 2012). Meta-analysis is able to increase statistical power through the use of a large number of data covering contrasted environmental conditions compared to single studies based on a limited number of sites, years, and replicates. Many meta-analyses of crop diversification focus on rotation-based diversification and cover crops (Beillouin et al., 2019), but a smaller number (12 vs. 36 for rotation) were published on intercropping (i.e. Pelzer et al., 2014; Yu et al., 2015; Raseduzzaman and Jensen, 2017; Martin-Guay et al., 2018).

Meta-analyses can be used to determine how intercropping affects the delivery of ecosystem services, given that supporting services, such as nutrient cycling, are necessary for the maintenance of all other ecosystem services (Millennium Ecosystem Assessment, 2005). The ecosystem functions supported by nutrient cycling are described as storage, internal cycling, processing and acquisition of nutrients (Costanza et al., 1997). Intercropping may increase both legume symbiotic $N_2$ fixation and cereal soil N acquisition in low-input systems, due to interspecific complementarity and facilitation (Jensen, 1996a; Hauggaard-Nielsen and Jensen, 2001; Bedoussac et al., 2015). However, to our knowledge, no quantitative synthesis has been performed to investigate how intercropping influences soil N uptake according to crop compositions and fertilization rates in temperate climates.

The aim of this study was thus to quantify and synthesise the effects of grain legume-cereal intercropping on the use of symbiotically fixed $N_2$ in legumes and soil-derived N (soil + fertilizer N) in cereals and legumes as compared to sole cropping in temperate agroecosystems. The overall effects were quantified using a meta-analysis based on data extracted from 29 published studies. We hypothesized that: (i) the amount of dinitrogen fixation in intercropped grain legumes is higher than in sole crop grain legumes, when expressed at equivalent density relative to sowing rates, (ii) intercropping increases the grain legume reliance on dinitrogen fixation (%N derived from the atmosphere), (iii) grain legumes in the intercrop acquire a lower amount of soil-derived N than in the sole crop at equivalent density, as a result of the competitive interactions between species, iv) intercropped cereal acquire a higher amount of soil-derived N than cereal sole crop at equivalent density due to competitive-complementarity interactions, and (v) the combined soil-derived N acquisition by the intercrop components is enhanced as compared to soil-derived N acquisition in both sole crops.

## 2. Materials and methods

### 2.1. Data collection

We searched for peer-reviewed literature using the Web of Science academic citation database (https://apps.webofknowledge.com) on 09 October 2017. The initial search equation was intercrop* AND (legum* OR pulse* OR faba* OR pea OR pisum OR lentil OR lupin OR soybean OR vetch OR vicia OR "common bean" OR phaseolus) AND (cereal OR barley OR wheat OR oat* OR maize OR triticale OR rye) AND (nitrogen OR N). This search returned 811 articles from which 222 articles reporting data from tropical climates were excluded. The remaining articles were screened for relevance at title, abstract and then full text levels using the following predefined selection criteria: (i) at least one intercropping between a cereal and a grain legume studied, (ii) written in English, (iii) based on field experiments conducted in a temperature climate latitude from $30-66^{\circ}$ and (iv) full-text articles were available. Eligible articles were additionally reviewed to check that the following data were reported: (v) $N_2$ fixation by the legume in intercropping and by the sole crop, (vi) total crop N uptake for both intercrop and sole crop treatments, and (vii) N fertilization rates when applied. If the same data were reported in multiple articles, they were only included once, from the article containing most detailed descriptions. References from selected articles were also reviewed to identify additional articles (seven articles were identified). We asked authors for raw data when an article presented averages from years and sites. For each study, an experimental unit was defined as the combination of site, year, cereal and legume crops, and agricultural practices (i.e. fertilization rate, sowing densities, and intercrop composition). Based on the selection criteria described above, our final data set was based on 29 articles (electronic supplementary material, Table S1) including results for 72 experiments and 207 experimental units. Data were extracted from figures and tables for each article using a web-based image analysis, Webplotdigitizer v4.1 (Rohatgi, 2018) or retrieved directly from the authors.

**Table 1**
Response variables used for the meta-analysis.

| Response variable | Comparison | Definition |
|---|---|---|
| % Ndfa | Intercrop legume/sole legume | Percent of N in the legume above-ground plant parts derived from $N_2$ fixation |
| Ndfa | Intercrop legume/sole legume | Amount of $N_2$ fixed by the legume in aboveground plant parts, kg $ha^{-1}$ |
| $Ndfs_{legume}$ | Intercrop legume/sole legume | Soil-derived N acquisition in aboveground plant parts kg $ha^{-1}$. $Ndfs_{legume}$ was calculated as the difference between total above-ground nitrogen and Ndfa (Hauggaard-Nielsen et al., 2009). |
| $Ndfs_{cereal}$ | Intercrop cereal/sole cereal | Soil-derived N acquisition in kg $ha^{-1}$. $Ndfs_{cereal}$ was equal to total crop N, whether or not fertilizer N was applied, i.e. fertilizer-derived N was included in the total soil N acquisition. |
| $Ndfs_{total}$ | Legume + Cereal intercrop/sole legume | Soil-derived N acquisition in aboveground plant parts, kg $ha^{-1}$ |
| | Legume + Cereal intercrop/sole cereal | Soil-derived N acquisition in aboveground plant parts, kg $ha^{-1}$ |

### 2.2. Response variables and moderators

The response variables included in the meta-analysis were dinitrogen ($N_2$) fixation (**Ndfa** and **% Ndfa)** in grain legumes (in the sole and intercrop aboveground biomass), and soil-derived N acquisition (**Ndfs**) in cereal and grain legume (in the sole and intercrop aboveground biomass), and the total soil-derived N in the intercrop aboveground biomass (Table 1). In addition, several characteristics of the study were included as moderators, to analyse their potential influence on the difference between intercrops and sole crops (Table 2): 1) intercropping design (physical species organization within the intercrop) 2) intercrop composition (proportion of each species in the intercrops at sowing), 3) crop species, 4) N fertilizer rates, 5) method used to calculate $N_2$ fixation, 6) management (conventional, organic and minimum tillage), 7) soil type, 8) precrop (preceding crop).

### 2.3. Statistical analyses

In each individual study, we quantified the magnitude of the intercropping effect on Ndfa and Ndfs in the aboveground biomass, relatively to the sole crops by calculating the log-transformed response ratio on normally distributed data:

$$\ln R = \ln\left(\frac{\overline{X}_{ic}}{\overline{X}_{sc}}\right) = \ln(\overline{X}_{ic}) - \ln(\overline{X}_{sc}) \quad (1)$$

where ($\overline{X}_{ic}$) is the mean value of the response variable (Ndfa or Ndfs) in intercropping and ($\overline{X}_{sc}$) is the mean value of the same response variable of the sole crop, considered as the control in the considered study (Hedges et al., 1999). The log ratio (Eq. 1) was computed for the total Ndfs in intercropping, and separately for Ndfa and Ndfs in inter- and sole cropped grain legumes and cereals. Total Ndfs in intercropping is calculated as the sum of Ndfs for the legume and Ndfs for the cereal in the intercrop. When comparing legume (or cereal) in intercropping versus legume (or cereal) in sole crop the variables Ndfa and Ndfs are measured in kg $ha^{-1}$. To be comparable, these variables were recalculated to equivalent plant density, compensating for the proportion of each crop type in the intercrop mixture sowing rates as:

$$\overline{X}_{ic} = \frac{\overline{X}_{ic}}{\rho} \quad (2)$$

where $\overline{X}_{ic}$ is the amount of N fixed by legume or acquired by cereal and legume in the intercrop mixture, and $\rho$ corresponds either to the proportion of cereal or legume in the mixture relative to the sowing rate as sole crop. No compensation for sowing density was made for % Ndfa, since this variable is independent of surface area, nor for total Ndfs which measures the total N acquisition per surface area regardless of species composition or density.

The log ratio (Eq. 1) was calculated for each study separately, together with its variance defined following (Hedges et al., 1999) as:

$$V_{\ln R} = \frac{(s_{ic})^2}{n_{ic}(\overline{X}_{ic})^2} + \frac{(s_{sc})^2}{n_{sc}(\overline{X}_{sc})^2} \quad (3)$$

where $\overline{X}_{ic}$ and $\overline{X}_{sc}$ are the mean values of the variables (Ndfa, % Ndfa and Ndfs) as in Eq. (1), $n_{ic}$ and $n_{sc}$ are the number of replicates in the considered study, and $s_{ic}$ and $s_{sc}$ are the within-study standard deviations. In several studies, $s_{ic}$ and $s_{sc}$ were not directly available and were derived from coefficient of variation and least significance differences (electronic supplementary material, methods A1). To facilitate

**Table 2**
Moderators extracted from the articles.

| Variable | Definition |
|---|---|
| Intercropping design | Two designs:<br>Additive design: each of the intercrop components are sown at equal or higher plant density than the sole crop; and<br>Replacement design: plant density of the sole crop has been replaced proportionally by the other component in the intercrop (Vandermeer, 1989). |
| Intercrop composition | Proportion of each species in the intercrops. % Legume-% Cereal, i.e. L50-C50. |
| Crop species | Crop species of legumes and cereals.<br>Legume species: common bean (*Phaseolus vulgaris*), faba bean (*Vicia faba*), lentil (*Lens culinaris*), lupin (*Lupinus angustifolius*), pea (*Pisum sativum*), and common vetch (*Vicia sativa*).<br>Cereal species: barley (*Hordeum vulgare*), oat (*Avena spp.*), rye (*Secale cereale*) and wheat (*Triticum spp.*). |
| N fertilization rates | The rates were grouped in four levels according to the amount of N fertilizer applied in the experiment:<br>- N0 = no fertilizer applied;<br>- N1 = 1 − 50 kg N per ha;<br>- N2 = 51 − 100 kg N per ha; and<br>- N3 > 100 kg N per ha |
| Method to quantify $N_2$ fixation | $^{15}N$ isotope dilution, $^{15}N$ natural abundance and difference method (Unkovich et al., 2008). |
| Management | Type of production: Conventional, organic, minimum tillage. |
| Soil type | Clay: clay and silty clay;<br>Fine loamy: clay loam, sandy clay loam and silt loam;<br>Coarse loamy: coarse loam, gravelly loam, loam and sandy loam; and<br>Sandy: sandy. |
| Precrop | Preceding crop. The crops were grouped in cereals, vegetables, oil crops, and others such as meadows. |

**Table 3**
Statistical models tested for the meta-analysis.

| Model | Fixed effect | Random effect | Weight |
|---|---|---|---|
| M1 | Null model | Article × common control × experimental unit | $V_{\ln R}$ corrected with variance-covariance matrix for multiple treatments |
| M2 | Null model | Article × common control × experimental unit | Simple imputation[b] |
| M3 | Null model | Article × common control × experimental unit | Variances derived from sample sizes |
| M4 | Null model | Article × common control × experimental unit | All variance equal to the mean of reported variance values |
| M5 | Moderator[a] | Article × common control × experimental unit | $V_{\ln R}$ corrected with variance-covariance matrix for multiple treatments |
| M6 | Moderator[a] | Article × common control × experimental unit | Simple imputation |
| M7 | Moderator[a] | Article × common control × experimental unit | Variances derived from sample sizes |
| M8 | Moderator[a] | Article × common control × experimental unit | All variance equal to the mean of reported variance values |

[a] Each moderator was tested separately in the meta-regression model. Moderators are listed in Table 2.
[b] Missing variance were set equal to the mean of existing variance values.

interpretation, log ratios were back-transformed to response ratios for the presentation of the results after statistical analyses.

Different models were used based on the availability of measures of variation. The first model was used for articles with complete data where the variances were reported. In order to be able to use all log ratios calculated in the articles, even when the variances were not reported, three other models were used. In these models, variances were imputed according to different methods to compare the effects of the moderator variables when different weights were used in the analysis (Table 3). Several studies included two or more experiments with the same control. Therefore, having the same control in the comparison with each treatment results in a statistically dependent log ratio. To avoid for this dependency, log ratios were grouped using a corrected covariance matrix. The matrix was calculated using the covariance_commonControl() function from the metagear package in the R software (Lajeunesse and Fitzjohn, 2015). A separate variance-covariance matrix was created for each of the tested moderators (model M5, fitted to a subset of the original data that included each of the moderators). In cases where the original article did not report any measure of variation (30–75 % of the articles, depending on the considered response variable; electronic supplementary material, Table S2a–c) different strategies were applied to account for the missing values:

- Simple imputation, where missing variance were set equal to the mean of existing variance values (based on all experimental units where standard deviations were reported in the original article, models M2, M6).
- Instead of using Eq. (3), variances were assumed to be proportional to

$$V_{\ln R} = \frac{n_{ic} + n_{sc}}{n_{ic}.\ n_{sc}} \quad (4)$$

  where $n_{ic}$ and $n_{sc}$ are the numbers of replicates for intercrop components and sole crops respectively (models M3, M7) (Hedges and Olkin, 1985; Koricheva et al., 2013).
- All variances were assumed equal to the mean of existing variance values (models M4, M8).

We estimated the weighted average of the individual log ratios for each response, separately. Heterogeneity of individual log ratios across studies was assessed using a $Q$ test. As the $Q$ test was highly significant, we used a mixed effect model instead of a fixed effect model for estimating the mean log ratio (Koricheva et al., 2013; Makowski et al., 2019). The weighted average of the individual log ratios was estimated by fitting a mixed-effect model to the whole data set by restricted maximum likelihood using the variances Eqs. (3) or (4) as weights. The significance of the difference between intercrop and sole crop was analysed by computing the 95 % confidence interval of the mean log ratio. We then assessed the influence of different moderators characterizing the intercrops and their environment (Table 3). For this purpose, each considered moderator was included as a categorical variable in a mixed effect model, and its effect on the log ratio was estimated. A moderator variable was considered to have a significant effect on the response variable when the $Q_M$-statistics for residual heterogeneity of moderators (omnibus test including all of the coefficients in the model) was significant ($p \leq 0.01$) (Viechtbauer, 2010). The strength of the effect of each moderator was also analysed by comparing the Akaike's Information Criterion (AIC) of the models fitted with and without each moderator (Lajeunesse, 2009; Makowski et al., 2019). Multiple comparisons (Tukey's Post hoc test) were performed to compare between moderator levels using a general linear hypothesis (glht) function from the R package multcomp (Hothorn et al., 2008). All analyses were performed with the rma.mv() function from the metafor package (Viechtbauer, 2010) in R (R Development Core Team, 2018).

### 2.4. Publication bias and sensitivity analysis

Funnel plots were used to explore evidence of publication bias (Philibert et al., 2012), which was then confirmed or rejected based on Egger's regression test (Egger et al., 1997; Sterne and Egger, 2006) including standard errors from log ratio as a predictor. Log ratios with a Cook's distances of more than four times the mean value were considered outliers (Viechtbauer and Cheung, 2010). The influence of outliers was then assessed by comparing fitted models with and without outliers (electronic supplementary material, Table S4a, b).

## 3. Results

### 3.1. Descriptive analysis

The data selected for our meta-analysis (electronic supplementary material, Table S1) came from studies that investigated grain legume-cereal intercropping in a wide range of agricultural regions in temperate climates. Many of the studies shared similar research objectives, looking at the effects of intercropping in comparison to their specific sole crop, with variations in sowing rates and proportions, legume crops or fertilization rates. The majority of the studies were based on replacement design and reported results of experiments conducted from 1981 to 2012. The most common grain legume used in the experimental units was pea (71 % of the experiments). Barley was the most frequently tested cereal (48 % of the experiments). Regarding intercrop composition, 61 % of the data set was based on 50 %-50 % mixtures. Most of the data sets came from intercropping systems carried out in Denmark ($n$ = 79 experiments), France ($n$ = 46), Germany ($n$ = 32) and Canada ($n$ = 25).

### 3.2. Intercrop increases fraction of $N_2$ fixed by grain legume

The meta-analysis based on a subset of 15 articles and 138 experimental units with reported variance (Eq. 3) showed no significant effect of intercropping on the amount of $N_2$ fixed by the legume based on equivalent density (Fig. 1a). When missing variances were estimated

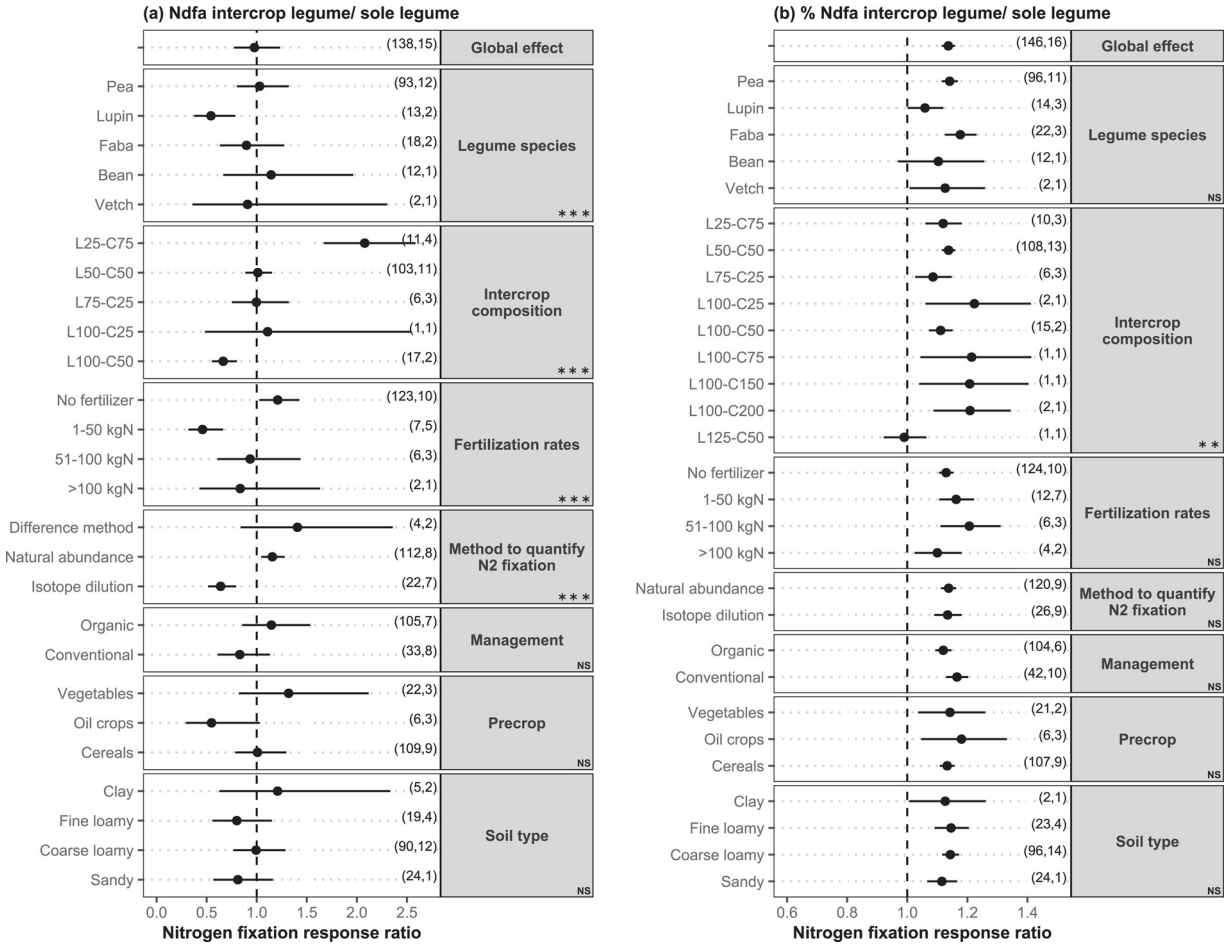


**Fig. 1.** Effect of intercropping on symbiotic $N_2$-fixation in grain legumes (response ratio). M1 and M5 models, mean estimates and 95 % confidence intervals for (a) amount of $N_2$ fixed by legume intercrop compared to legume sole crop at equivalent density relative to sowing rates and (b) percent of Ndfa of legume intercrop compared to legume sole crop. Negative values indicates a negative effect of intercropping on $N_2$ fixation. Note differences in scale on the *x* axis between figures. Numbers in parenthesis show number of experimental units and articles, respectively.

(see 2.3 Statistical Analyses), the number of articles and experimental units increased to 26 and 207, respectively, and the analysis based on this larger data set showed a small reduction on the overall effect of Ndfa in the intercropped legumes compared to the legume sole crops (response ratios from 0.85 to 0.90, depending on the strategy used to compensate for missing variance; electronic supplementary material, Table S2a). This reduction was statistically significant only when using the model M4 (i.e. when all variances were set equal to the mean of reported variances; electronic supplementary material, Table S2a). The moderators that significantly influenced the Ndfa response to intercropping were intercropping composition, legume species, fertilization rates and method used to quantify $N_2$ fixation ($p \leq 0.01$, Fig. 1a, electronic supplementary material, Table S3a). These moderators had significant effects also when using the total data set (model M6, electronic supplementary material, Tables S3a, S5a). There was a strong difference in Ndfa between the intercrop composition L25-C75 and the other compositions, as well as between L50-C50 and L100-C50 (Fig. 1a). Moreover, there were significant pairwise differences within legumes species on Ndfa, having strongest effects on pea compared to lupin (+1.90, 95 % CI = [1.25, 2.87], $p < 0.001$) and faba-lupin (+0.60, 95 % CI = [0.37, 0.98], $p = 0.03$) according to post hoc test. Intercropping had a more negative effect on Ndfa when N fertilizers were applied than under zero fertilization, but the difference was significant only between zero-N and the application of $1-50$ kg N $ha^{-1}$ (+0.38, 95 % CI = [0.22, 0.64], $p < 0.001$, post hoc test). With respect to the method used to quantify $N_2$ fixation, there was a significant difference between natural abundance and isotope dilution methods, with a negative effect of intercropping on Ndfa, when the isotope dilution method was used.

There was a significant overall positive effect of intercropping (+14 %, 95 % CI = [11, 16]) on % Ndfa (Fig. 1b, electronic supplementary material, Table S2a). When using the simple imputation variance, the intercropping effect was slightly larger and still significant (+16 %, 95 % CI = [11, 20], model M2, electronic supplementary material, Table S2a). Intercrop composition was the only moderator that significantly influenced the effect of intercropping on % Ndfa, with less positive effects in most cases where the legume component was in majority of the mixture (Fig. 1b, electronic supplementary material, Table S3b). The effect of intercrop composition was significant also when using the whole data set with simple imputation to compensate for missing variances, and in this analysis, legume species was also a significant moderator (models M2, M6, electronic supplementary material, Table S5a).

Several outliers were found in the Ndfa data sets (electronic supplementary material, Table S4a). For the amount of Ndfa, the response ratios varied from 0.96 to 1.12 when each of the outliers was removed. In the case of % Ndfa, the response ratios remained the same when removing outliers in comparison to the value obtained with the whole data set (electronic supplementary material, Table S4a). There was no effect of removing outliers when each of the moderators was included in the model. A significant publication bias was identified for the Ndfa ($z = 3.4$, $p < 0.001$) but not for the analysis of % Ndfa (electronic

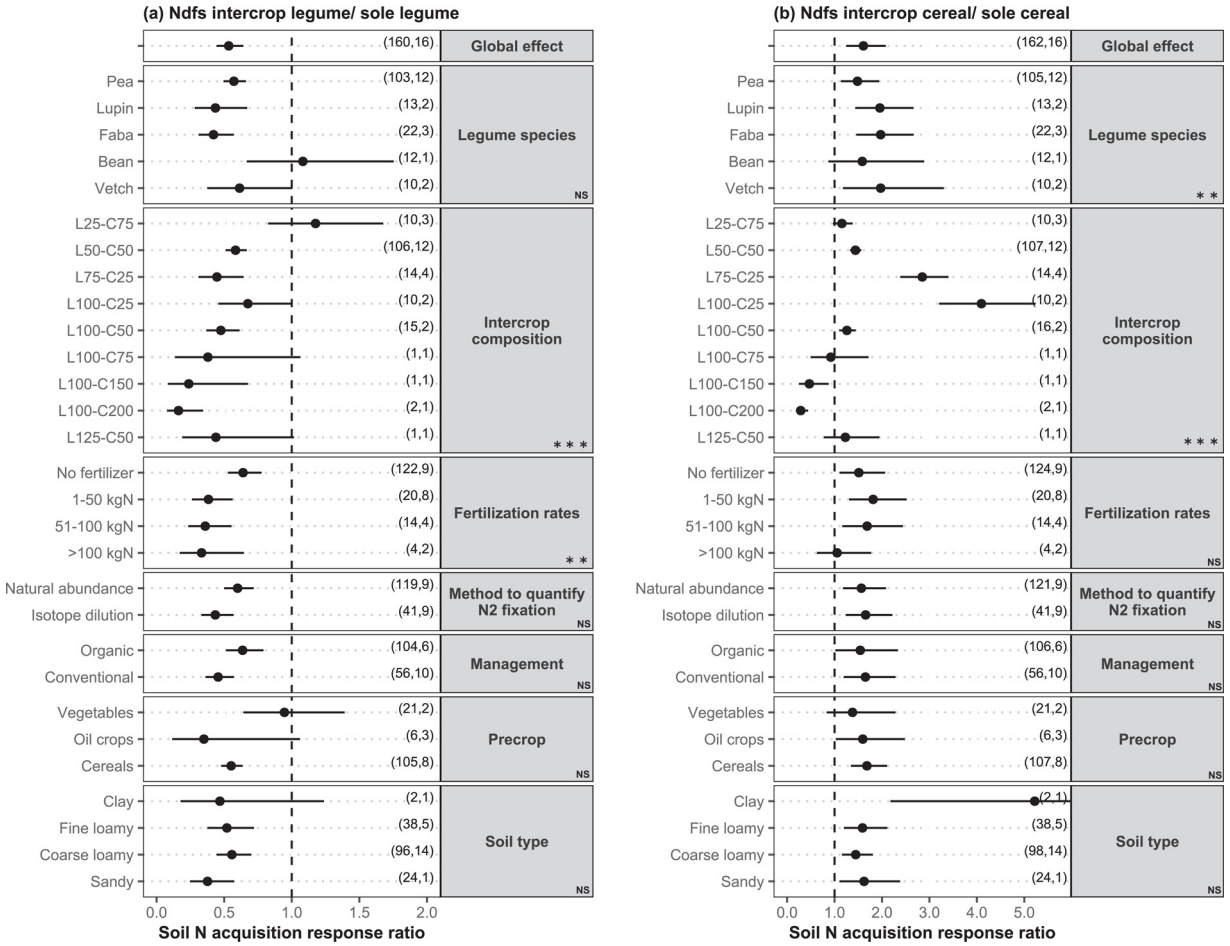


**Fig. 2.** Effect of intercropping on soil N acquisition (response ratio) at equivalent density relative to sowing rates. M1 and M5 models, mean estimates and 95 % confidence intervals for (a) legume intercrop compared to legume sole crop and (b) cereal intercrop compared to cereal sole crop. Negative values indicates a negative effect of intercropping on soil N acquisition. Note differences on scale in the *x* axis between figures. Numbers in parenthesis show number of experimental units and articles, respectively.

supplementary material, fig. S1a, 1b).

### 3.3. Soil N acquisition

#### 3.3.1. Use of soil N resources by the intercropped compared to sole crop

Overall, there was a 47 % decrease in soil N uptake by the intercropped legumes (+0.53, 95 % CI = [0.44, 0.64], $n$ = 160) compared to sole crop legumes, expressed at equivalent density (Fig. 2a). The response ratio decreased to +0.42 (95 % CI = [0.33, 0.53], $n$ = 200), when the whole data set was included with simple imputed variance, corresponding to a 58 % reduction of the soil N acquisition in intercropped legumes compared to sole crop legumes (electronic supplementary material, Table S2b). The fertilization rate was affecting the response ratio of Ndfs, with more negative effects of fertilized intercropping than unfertilized ones (Fig. 2a). However, the N fertilization moderator was slightly significant between zero-N and the N levels lower that 100 kg N $ha^{-1}$ of N fertilization (Fig. 2a). Based on the simple imputation to compensate for missing variances fertilization rate was not a significant moderator when the whole data set was used (model M6, electronic supplementary material, Table S5b). Intercrop composition (showing strong effect between L25-C75 and respectively L50-C50, L75-C25, L100-C50 and L100-C200) was also significant moderator for the difference in legume soil acquisition between intercrop and sole crop (model M5, electronic supplementary material, Table S3c).

Intercropping had a positive effect on soil N uptake by cereal crop as compared to cereal sole crops (+1.61, 95 % CI = [1.24, 2.08], $n$ = 162) (Fig.2b, electronic supplementary material, Table S2c). This means that cereals acquired on average 61 % more N from soil and fertilizer N resources, when intercropped with legumes than when grown as cereal sole crops (expressed at equivalent density). When including a higher amount of studies (using simple imputation to control for missing variance, Model M2), the response ratio decreased slightly, but was still significant (+1.53, 95 % CI = [1.20, 1.96], $n$ = 200) (electronic supplementary material, Table S2c). The effect of intercropping on soil N uptake in the cereal depended on intercrop composition and legume species in the intercrop. Studies with less proportion of cereal plants in the intercrop showed a stronger effect. Furthermore, legume species in the intercrop acquires greater amount of soil-derived N when the cereal was intercropped with faba (+0.75, 95 % CI = [0.60, 0.94], $p$ = 0.006, post hoc test) and lupin (+0.76, 95 % CI = [0.60, 0.96], $p$ = 0.010, post hoc test) in comparison with the pea. Using the simple imputation variance data set did not change the significance of the moderators for the response variable cereal soil N acquisition (electronic supplementary material, Table S5b).

Outliers were detected also in the Ndfs data sets. When removing the outliers, the Ndfs response ratios ranged from +0.51 to +0.59 for legumes; and from +1.46 to +1.63 for cereals, which is very close to the original (overall) response ratio of +0.53 and +1.61 for legumes and cereals respectively. Funnel plots and regression tests showed no sign of publication bias for the N soil uptake data set, in neither legumes ($z$ = 1.3, $p$ = 0.19) nor cereals ($z$ = 0.8, $p$ = 0.42) (electronic

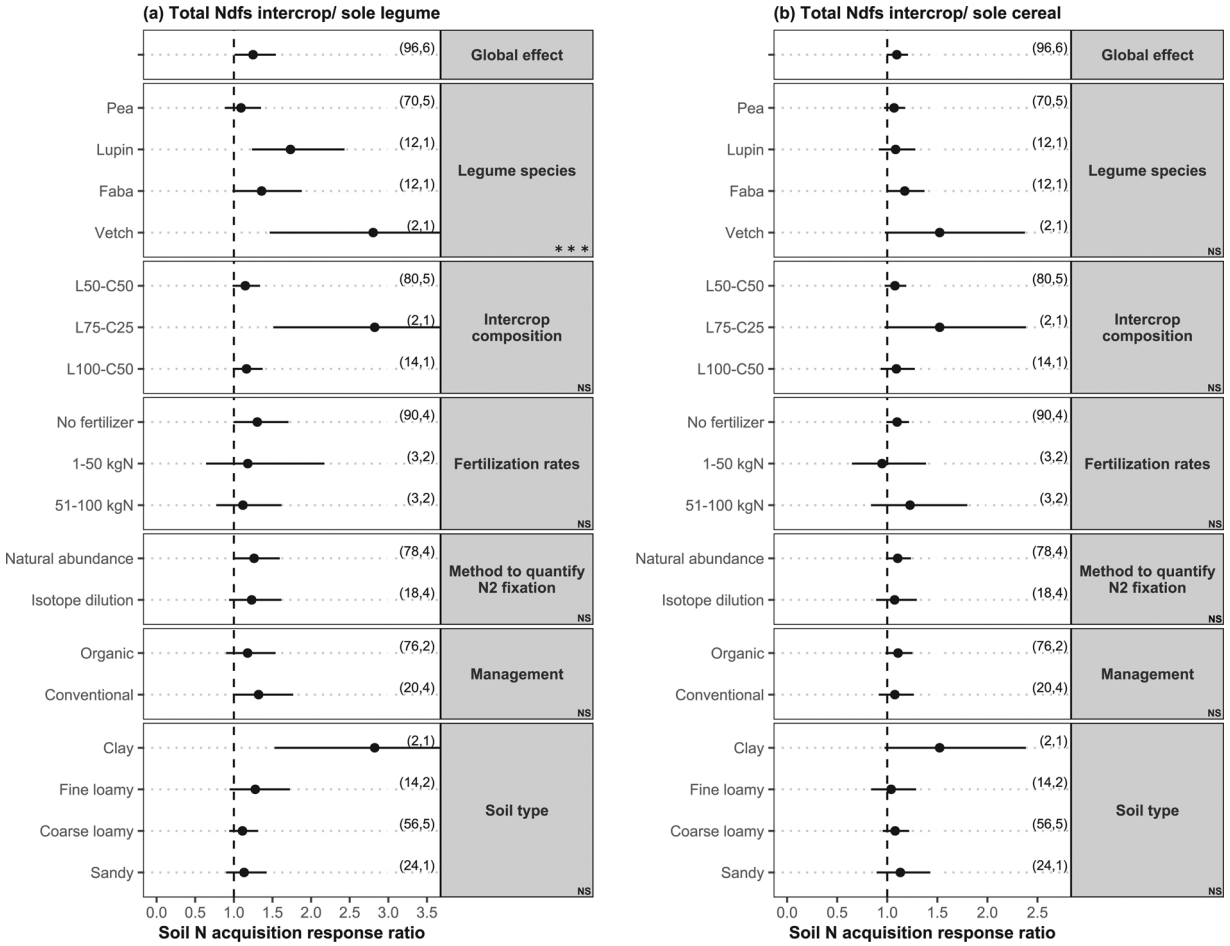


**Fig. 3.** Effect of intercropping on soil N acquisition (response ratio). M1 and M5 models, mean estimates and 95 % confidence intervals for (a) legume + cereal intercrop compared to legume sole crop and (b) Ndfs in legume + cereal intercrop compared to cereal sole crop. Negative values indicates a negative effect of intercropping on soil N acquisition. Note differences in scale on the *x* axis between figures. Numbers in parenthesis show number of experimental units and articles, respectively.

supplementary material, fig. S1c, 1e).

### *3.3.2. Comparison of total soil-derived N in the intercrop compared to sole crops*

The total soil N uptake (legume + cereal) in intercrops was on average 25 % higher than the soil N acquisition in legume sole crops (+1.25, 95 % CI = [1.01, 1.54], $n = 96$) (Fig. 3a, electronic supplementary material, Table S2b). When increasing the number of observations by simple imputation, the response ratio showed an increment of 14 %, indicating a greater soil N uptake by the legume-cereal mixture (electronic supplementary material, Table S2b). Legume species influenced the effect of intercropping on total soil N uptake (having greater effect in the intercrops including lupin, vetch and faba bean), when tested with simple imputation to account for missing variance (Model M6, electronic supplementary material, Table S5b). When using the variance-covariance matrix, also intercrop composition was significant moderator for the difference in total soil N uptake between intercrops and legume sole crops (Model M5, Fig. 3a, electronic supplementary material, Table S5b).

Although, the response ratio was not significant (+1.09, 95 % CI = [0.99, 1.21], $n = 96$), total Ndfs was 9% greater in intercrops (sum of Ndfs in the legumes and cereals) than in cereal sole crops (Fig. 3b, electronic supplementary material, Table S2c), averaged across all studies. None of the moderators (Fig. 3b, electronic supplementary material, Table S5b) significantly influenced the effect of intercropping on total soil N uptake compared to cereal sole crops. When increasing the data set by simple imputation to control for missing variance, total Ndfs was 4% lower in intercropping than in cereal sole crops (electronic supplementary material, Table S2c), but this was not significant.

We did not account for outliers when comparing total Ndfs and sole crops due to small data set. No evidence of publication bias was founded for the total Ndfs in comparison to the sole legume crop ($z = 0.3$, $p = 0.76$). However, when comparing total Ndfs to the sole cereal crop, we identified signs of publication bias ($z = 2.3$, $p = 0.02$), (electronic supplementary material, fig. S1d, 1f).

## 4. Discussion

### *4.1. Competition, complementary and facilitation interactions of grain legume-cereal intercropping*

This meta-analysis summarized the effects of intercropping on the efficiency in crop N acquisition, focusing on the $N_2$ fixation and soil N acquisition. In average across studies, the meta-analysis resulted in a 13–16 % (depending on model; electronic supplementary material Table S2a) increase of the % N derived from symbiotic $N_2$ fixation in intercropped legumes compared to legume sole crops. This result demonstrates that intercropping consistently increases the reliance on $N_2$ fixation. Intercropping stimulates complementary N use between legumes and cereals by increasing legume dependency on $N_2$ fixation and increasing cereal soil N acquisition based on equivalent density relative to sowing rates. Gaba et al. (2015) described how cereal-legume

intercropping improves N use during the growing season, due to complementarity functioning in which a higher proportion of the available soil N is recovered by the cereal. For instance, with low soil N availability, intercropped wheat plants benefit from a higher soil N acquisition compared to sole crop wheat plants. Cereals are stronger competitors for inorganic N (Jensen, 1996b; Hauggaard-Nielsen and Jensen, 2001) thus, forcing legumes to depend to a higher degree on symbiotic $N_2$ fixation (Hauggaard-Nielsen et al., 2009; Bedoussac and Justes, 2010). By reducing the plant-availability of soil mineral N, the cereal will also contribute to reduce the inhibitory effect of mineral N on the symbiotic $N_2$ fixation process (Jensen, 1986). When comparing the legume species, faba bean relied 20 % more on $N_2$ fixation (% Ndfa) in intercropping, with an average of 83 % N derived from $N_2$ fixation, followed by peas and common beans, with 19 % and 12 % respectively. Although the inhibitory effect of soil mineral N on $N_2$ fixation was reduced by the cereal competition for soil mineral N, the decrease in the amount of symbiotic $N_2$ fixation in intercropping (recalculated to compensate for different sowing rates, i.e. expressed on an equivalent density based on sowing rates) was expected, considering that Ndfa can be affected by competition from the cereal for several growth resources, such as light, non-nitrogen nutrients and water (Jensen, 1996b; Hauggaard-Nielsen et al., 2009). Bedoussac et al. (2015) found that a reduction in the amount of $N_2$ fixed by the intercropped legume was due to a decrease in the intercropped legume biomass in comparison to the sole crop legume. In the studies using a replacement design, the intercropping effect on the amount of $N_2$ fixed by the legumes were close to zero or positive. This may be because replacement designs reduce the interspecific competition, promoting complementarity which may enhance the yield per plant (Iverson et al., 2014). Moreover, the studies vary in plant densities (depending on sowing recommendations for the specific location), design, initial soil N and even legume species. Notably, in studies using L25-C75, the amount of $N_2$ fixed by the legumes were more positively influenced by intercropping than in the other relative frequencies of intercrops. In contrast, in studies with additive design (L100-C50), the reduction in the amount of $N_2$ fixation in response to intercropping may be reflected by the slight reduction in % Ndfa in comparison with L25-C75 and L50-C50. In addition, the amount of $N_2$ fixed in intercropped legumes was increased compared to sole crop legumes for the studies without application of N fertilizers, but not with N fertilizer. This indicates that application of N fertilizers inhibits the capacity of the legume to fix atmospheric nitrogen directly, in line with studies where the potential of $N_2$ fixation and nodule development were reduced by increasing N fertilization levels (Jensen, 1996b; Andersen et al., 2005; Li et al., 2009a). However, the reduction may also be indirect, via increased competition from the cereal for other growth resources, e.g. light, due to the N-fertilization.

The results of our meta-analysis demonstrated that the soil derived N acquisition by the intercropped legumes decreased compared to the legume sole crop (between 47 % and 58 %) when expressed at equivalent density. This decrease was influenced by the intercrop composition and fertilization rate. For example, when the intercrop includes 25 % of legume (L25-C75) there was a positive effect on soil N acquisition as compared to the sole crop legumes. Moreover, in studies where low level of N fertilizer was applied, the soil + fertilizer N acquisition by the intercropped legumes were less strongly reduced than in the overall effect that included all fertilizer treatments. Studying several plant densities and fertilization rates, Corre-Hellou et al. (2006) found a decreased soil-derived N (soil + fertilizer N) uptake per plant when the pea was intercropped, and even more when there was an increase in the soil N supply. The reduction in the soil N acquisition by the intercropped legumes due to poor competitive ability of the legume has also been reported by Jensen (1996b); van Kessel and Hartley (2000); Ghaley et al. (2005).

Across studies, the cereal soil N acquisition was 54–64 % higher in intercrop than in the sole crops (fertilization rates were the same in the intercropping and sole crops), when expressed at equivalent density and using the model M1 with the covariance matrix. Only one study, had different fertilization rates between the intercrop and sole crop when using the whole data set. Intercrop composition and legume species in the intercrop had a significant effect on the overall effect of intercropping on the soil-derived N uptake. Intercropped cereals usually accumulate a greater amount of soil N per plant, as reported by several studies (Corre-Hellou et al., 2006; Hauggaard-Nielsen et al., 2009). For example, Naudin et al. (2010) observed that the cereal had a higher N demand than the legume during the vegetative phase, resulting in an increased dominance by the cereal, for the soil mineral N uptake when intercropped with grain legumes. The meta-analysis results also show that a lower cereal plant density increases the positive effect of intercropping on the cereal N acquisition (Fig. 2b). This is likely due to the reduction in the interspecific competition for soil N in low cereal plant densities, as well as greater access to soil N thanks to intra-specific complementarity as legumes increase their reliance on $N_2$ fixation in intercropping (Bedoussac and Justes, 2010). Additionally, there was a significant difference between the cereals intercropped with pea, lupin and faba bean, where cereals intercropped with pea showed a smaller response in soil N acquisition than cereals intercropped with lupin and faba bean, probably linked to the higher amount of $N_2$ fixed by the pea in comparison to the lupin and faba bean. Interestingly, fertilization rates did not show a significant effect on soil N uptake. In a previous meta-analysis, Yu et al. (2015) showed that land equivalent ratio (LER) was reduced when N fertilization was applied (i.e. 100 kg N $ha^{-1}$). This negative effect of N fertilizer on LER was attributed to the increase of interspecific competition and the reduction of symbiotic $N_2$ fixation. Similar results reported that LER could be reduced with higher levels of N fertilizer, as shown by Bedoussac and Justes (2010) for wheat- pea intercrops, due to a strong interspecific competition with high N availability. Pelzer et al. (2014) argued that although the N fertilization rate did not have a significant effect on the total LER, there was a large variability among the studies included in their meta-analysis.

The total soil-derived N in the intercrop was 25–39 % greater than in the sole crop legume (Fig. 3a).This result was strongly affected by the legume species. In contrast, the total intercrop and cereal sole crop Ndfs were not significantly different. Thus, it is possible to conclude that these lacks of effects are due to the strong competition for available resources between the cereal and legume throughout the similar growth period (Hauggaard-Nielsen et al., 2009). Clearly, the balance between complementary and competition in cereal-legume mixtures may depend on environmental conditions (Duchene et al., 2017), soil N availability, and lastly, plant growth dynamics, as described by Hauggaard-Nielsen et al. (2001) where the intercropping promote the root growth in the intercropped barley.

The method used to quantify $N_2$ fixation influenced the amount of $N_2$ fixed by the intercropped legumes. The negative effect of intercropping on Ndfa in results obtained with the $^{15}N$ isotope dilution method compared to results from the natural abundance method on Ndfa could be an effect of additional N fertilization due to the application of $^{15}N$-enriched N to the soil in the isotope dilution method. This additional soil N would primarily benefit intercropped cereals, which then reduce legume growth and $N_2$ fixation due to competition for light, water and other nutrients than N. However, these differences cannot be determined by the current meta-analysis, due to large variation in the amount of fertilizer applied (ranging from 5 to 90 kg N $ha^{-1}$ among the studies using the isotope dilution method). Also differences in the fertilization recoveries between crop species and pedo-climatic conditions (Witty and Giller, 1991) may have influenced these results. In results obtained with the N difference method, there was a large variation in the effect of intercropping on Ndfa (Fig. 1). This method is associated with large uncertainties, with risks of underestimating Ndfa in legume sole crops and overestimations in intercrops (Carlsson and Huss-Danell, 2003). Differences in N acquisition and rooting pattern between grain legumes and cereals (Jensen, 1996b) could potentially cause further variations in the effect of intercropping depending on the method used

to quantify $N_2$ fixation.

Most of the studies included in this meta-analysis did not report details about specific species used as precrop before the experiments, which is why this moderator was based on a few groups of precrops. Management and precrop did not influence the effect of intercropping on any of the response variables.

### 4.2. Potential of intercropping to diversify cropping systems

Our study confirms previous findings, and highlights that intercropping enhance nitrogen use efficiency in crop stands through the increased acquisition of soil derived nitrogen in the cereal, and stimulate the reliance on symbiotic nitrogen fixation in the grain legume. Our meta-analysis included a wide range of temperate climate conditions, soil types, plant densities and grain legume species, providing a robust assessment of intercropping ability to increase complementarity effects on the efficient use of resources in space due to positive interspecific interactions (Tilman et al., 1997; Loreau, 2000; Cardinale et al., 2006). Furthermore, there is a potential for diversified cropping systems via intercropping to produce cereal and grain legumes, while increasing the use of N-sources and reduce external inputs such as N fertilizers. Bybee-Finley and Ryan (2018) emphasised that the focus of intercropping may be to perform equally as the sole crop when enhancing ecosystem services instead of pointing to transgressive overyielding (total intercrop yield is higher than the most productive of the component sole crops). This is in accordance with a focus on achieving complementarity and facilitation interactions through intercropping, stimulating more efficient N use as shown in this meta-analysis and making it possible to reduce the global inputs of N fertilizers as suggested by Jensen et al. (2020). Various factors such as plant density, design and intercrop composition should be considered to regulate the interaction between diversity of crop species, and maximize crop growth (Trenbath, 1976; Vandermeer, 1989; Hauggaard-Nielsen et al., 2006).

### 4.3. Limitations of the study

We limited our meta-analysis to focus on studies where the intercropped species where sown at the same time. Our analysis did not include any temporal niche differentiation study such as relay intercropping, limiting different cereal and grain legumes species used in the intercropping systems. A large number of studies were based on studies using peas in the intercrop component, therefore, one needs to be cautious when the results are generalized to other grain legumes. Moreover, many studies included in this meta-analysis did not provide any information on actual plant densities (total number of plants emerged) in the field experiments. Thus, a compensation based on different sowing rates was necessary to compare intercropped species with their respective sole crop on an equivalent density basis. Furthermore, initial soil N or total N derived from N fertilizer when a fertilizer was applied were either not reported, thus constraining our study in the understanding of the factors that affect the dynamics in the N use complementarity in the intercropping systems.

Despite the small effects of outliers on the estimates, there is no a clear explanation for the publication bias in the studies included for quantifying the amount of $N_2$ fixation and total Ndfs compared to sole cereal crop. Some of the possible reasons for this bias may be due to a small number of studies included in the analysis but also due to the preferential publication of significant results compared to non-significant results (Rothstein et al., 2006; Makowski et al., 2019)

## 5. Conclusion

This meta-analysis quantified the effect of intercropping of grain legumes-cereals on the symbiotic $N_2$ fixation and soil-derived N. These results highlight that the amount of $N_2$ fixation in intercropped grain legumes was decreased compared to sole crop grain legumes. The extent of this reduction was variable, depending on other factors such as intercrop composition, legumes species, fertilization rates and method used to quantify $N_2$ fixation. Indeed, the reduction in the amount of $N_2$ fixation in intercropped grain legumes could be a consequence of the reduction of legume biomass due to interspecific competition with the cereal. The results of our analysis also indicate that intercropping increased the grain legume reliance on the symbiotic $N_2$ fixation. Furthermore, these results suggest that intercropping consistently stimulate complementary N use between legumes and cereals. Grain legumes in the intercrop acquired a lower amount of soil-derived N than in the sole crop while the cereals in the intercrop acquired a higher amount of soil-derived N than in the sole crop. However, the combined soil-derived N acquisition by the intercrop components was enhanced only when compared to soil-derived N acquisition in the legume sole crop. Currently, millions of hectares of grain legumes are sole cropped globally. The grain legumes use soil mineral N derived from mineralization of soil organic matter and reduces its symbiotic $N_2$ fixation. Potentially this soil mineral N could be used by non-fixing crop species. The results of the meta-analysis show that there is a great opportunity for improving the efficiency in using soil-derived mineral N/fertilizer N in global cropping systems by intercropping cereals and grain legumes, due to the uneven sharing of the mineral N as a result of competitive ability of the cereal for mineral N, while at the same time stimulating symbiotic $N_2$ fixation in the legume.

## Data accessibility

Datasheet of all observations included in meta-analysis will be available in Mendeley data.

## CRediT authorship contribution statement

**Carolina Rodriguez:** Conceptualization, Methodology, Formal analysis, Writing - original draft. **Georg Carlsson:** Conceptualization, Supervision, Writing - review & editing. **Jan-Eric Englund:** Formal analysis, Methodology, Writing - review & editing. **Adam Flöhr:** Formal analysis, Methodology. **Elise Pelzer:** Conceptualization, Supervision, Writing - review & editing. **Marie-Hélène Jeuffroy:** Conceptualization, Writing - review & editing. **David Makowski:** Conceptualization, Methodology, Formal analysis, Writing - original draft. **Erik Steen Jensen:** Conceptualization, Supervision, Writing - review & editing.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eja.2020.126077.

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