



# Method for estimating nitrogen input by symbiotic fixation on Swiss farms

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## Abbreviation

<i>AGN</i>	Above-ground nitrogen in current crop
<i>ANF</i>	Asymbiotic nitrogen fixation
<i>BGN</i>	Below-ground nitrogen in current crop
<i>BGNF</i>	Below-ground nitrogen factor
<i>BGTNF</i>	Below-ground and transfer nitrogen factor
<i>BNF</i>	Biological nitrogen fixation
<i>DM</i>	Dry matter
<i>Leg<sub>RA</sub></i>	Relative abundance of legumes (dt DM legumes dt <sup>-1</sup> DM total biomass)
<i>NHI</i>	Nitrogen harvest index
<i>N<sub>con</sub></i>	N content in the legume fraction of the above-ground biomass (kg N dt <sup>-1</sup> DM)
<i>N<sub>dfr</sub></i>	N derived from rhizodeposition
<i>N<sub>fert</sub></i>	Amount of plant-available nitrogen applied in fertilizers (kg N ha <sup>-1</sup> )
<i>N<sub>fix</sub></i>	Amount of symbiotically fixed nitrogen (kg N ha <sup>-1</sup> )
<i>N<sub>root</sub></i>	Amount of nitrogen in the roots (kg N ha <sup>-1</sup> )
<i>N<sub>stubble</sub></i>	Amount of nitrogen in stubbles or straw (kg N ha <sup>-1</sup> )
<i>N<sub>y</sub></i>	Nitrogen yield (harvested products removed from the field) (kg N ha <sup>-1</sup> )
<i>PN<sub>dfa</sub></i>	Proportion of nitrogen derived from the atmosphere
<i>SB</i>	Suisse-Bilanz (Swiss farm balance system)
<i>SNF</i>	Symbiotic nitrogen fixation
<i>Y<sub>DM</sub></i>	Harvested dry matter yield (dt DM ha <sup>-1</sup> )

## Summary

Farm-gate nitrogen (N) balances consider all inputs to a farm and outputs from it in the form of agricultural products. In many approaches of farm-gate N balances, inputs through biological nitrogen fixation (*BNF*) are not included because of difficulties in quantifying these pathways. If inputs through *BNF* are considered, they are usually estimated by simple empirical relationships or by standard values. At farm level, symbiotic nitrogen fixation (*SNF*) usually contributes by far the most to *BNF* because only small N quantities per hectare of agricultural land are fixed by asymbiotic nitrogen fixation, the other pathway of *BNF*. This study aimed at developing the basis for farm-specific estimation of the amount of N annually entering the farm system via *SNF*. Such would allow to include *SNF* in a farm-gate N balance of Swiss farms. The estimation method should be applicable to permanent and temporary grasslands, grain legumes and cover crops, and should be able to cope with the limited data availability on farms.

In a literature review, two empirical models for estimating symbiotically fixed nitrogen were selected, one for grassland systems and one for annual grain legumes. In both models, the amount of N in the harvested products, which is related to the yield of any given crop, is used as a pivotal input parameter. The selected models were adapted in order to better represent Swiss production systems and to make use of readily available farm data.

The model for grassland systems is based on nine input parameters. The estimations of N inputs by *SNF* obtained with this model fit well with data from Swiss experiments. However, the estimates are subject to great uncertainty because of the difficulty to specify the input parameters for the specific farm conditions and management accurately. For five of the input parameters, standard values based on extensive literature research are proposed either because the imprecision caused by using these standard values is reasonably small or no feasible alternatives could be identified. Thus, these variables are considered as model constants and do not need to be determined for specific farms. Three other parameters must be farm-specific and can be approximated from farm data on the intensity of utilization of the grassland area (herbage yields, level of fertilization, and N content of the legumes at harvest). These parameters can be determined with a similar amount of work as required for the current legally prescribed farm nutrient balance (Suisse-Bilanz). The last input parameter, the relative abundance of legumes, has a great influence on the estimation of the amount of N fixed by grassland and its accuracy. It can currently neither be approximated from already available farm data nor from remote sensing, and its collection in the field requires a large amount of work. As a trade-off between accuracy and required amount of work, we propose to visually categorize the grasslands into six different classes of legume relative abundances. This approach does not substantially decrease accuracy for the legume relative abundances classes below 15%, which cover most of the permanent grasslands. However, for the classes with 30 to 75% legumes, the predicted *SNF* could deviate by about 100 kg N ha<sup>-1</sup>.

The model for grain legumes is based on six input parameters and allows a sound estimation of *SNF* using the N uptake of the whole crop (above- and below-ground plant parts). N uptake can be estimated from the crop yield determined by farmer. An important parameter is the N harvest index. In future, it could be derived from the Swiss variety testing program and would thus allow a variety-specific estimation of *SNF*. For the other parameters, which are crop-specific, standard values based on the literature are proposed. The accuracy of the estimated amount of N fixed strongly depends on the determination of crop yield.

For cover crops, only few data on *SNF* are available in the literature. For clover-grass mixtures used as cover crops, values based on Swiss literature can be adopted. However, the results are considered rather inaccurate due to the rough estimation of both yield and legume relative abundance. For pure legume and legume-non legume cover crops, bibliographic data are too scarce to propose a reliable estimation method.

## Zusammenfassung

Die Hoftor-Bilanz für Stickstoff (N) umfasst alle Inputs in einen Landwirtschaftsbetrieb und die Outputs über die landwirtschaftlichen Produkte. In vielen Berechnungsansätzen wird bei der Hoftor-Bilanz jedoch der Input durch die biologische Stickstoff-Fixierung (*BNF*) nicht berücksichtigt, da es schwierig ist, diesen Pfad zu quantifizieren. Bei Methoden, die die *BNF* berücksichtigen, wird dieser Input gewöhnlich mit Hilfe von Standardwerten oder empirischen Formeln geschätzt. Auf Betriebsebene trägt die symbiotische Stickstoff-Fixierung (*SNF*) in der Regel bei weitem am meisten zur *BNF* bei, weil nur geringe N-Mengen pro Hektare landwirtschaftliche Nutzfläche durch die asymbiotische Stickstoff-Fixierung, den anderen Pfad der *BNF*, fixiert werden. Das Ziel dieser Studie war, die Grundlagen für eine Methode zur betriebsspezifischen Schätzung der N-Menge, die jährlich über die *SNF* in die landwirtschaftlichen Betriebe gelangt, zu entwickeln, damit dieser Input in der N-Bilanz der schweizerischen Landwirtschaftsbetriebe berücksichtigt werden kann. Die Methode soll für Natur- und Kunstwiesen, Körnerleguminosen sowie Zwischenkulturen anwendbar sein und soll die beschränkte Datenverfügbarkeit auf Landwirtschaftsbetrieben berücksichtigen.

In einer vertieften Literaturrecherche wurden zwei empirische Modelle zur Schätzung des symbiotisch gebundenen Stickstoffs ausgewählt, eines für Grasland sowie eines für einjährige Körnerleguminosen. In beiden Modellen wird die N-Menge in den Ernteprodukten, die mit dem Ertrag einer bestimmten Kultur in Beziehung steht, als wichtiger Eingabeparameter verwendet. Die ausgewählten Modelle wurden modifiziert, damit die schweizerischen Produktionssysteme besser abgebildet und landwirtschaftliche Betriebsdaten verwendet werden können.

Das Modell für Grasland basiert auf neun Eingabeparametern. Die mit diesem Modell erhaltenen Schätzungen der *SNF* stimmen gut mit schweizerischen Versuchsergebnissen überein. Die Schätzungen sind jedoch mit grossen Unsicherheiten behaftet, weil es schwierig ist, bei den Eingabeparametern die betriebsspezifischen Verhältnisse und die Bewirtschaftung exakt einzubeziehen. Für fünf Eingabeparameter werden Standardwerte, die aus der Literatur abgeleitet wurden, vorgeschlagen, da entweder die Schätzgenauigkeit nur wenig durch die Verwendung dieser Standardwerte reduziert wird oder da keine praktikablen Alternativen gefunden werden konnten. Diese Parameter werden deshalb als Modellkonstanten betrachtet und müssen nicht für die einzelnen Betriebe bestimmt werden. Drei weitere Parameter müssen betriebsspezifisch sein und können mit Hilfe von Betriebsdaten zur Nutzungsintensität des Graslands (Wiesenerträge, Düngungsniveau und N-Gehalt der Leguminosen bei der Ernte) geschätzt werden. Diese Parameter können mit einem ähnlichen Arbeitsaufwand erhoben werden, wie er für die derzeit gesetzlich vorgeschriebene betriebliche Nährstoffbilanz (Suisse-Bilanz) erforderlich ist. Der letzte Eingabeparameter, der Anteil der Leguminosen an der Biomasse des Bestands, hat einen grossen Einfluss auf die Schätzung der fixierten N-Menge und deren Genauigkeit. Er kann zurzeit weder aus bereits vorhandenen Betriebsdaten noch aus der Fernerkundung abgeleitet werden und seine Erhebung im Feld erfordert einen grossen Arbeitsaufwand. Als Kompromiss zwischen der Genauigkeit und dem erforderlichen Arbeitsaufwand schlagen wir vor, das Grasland visuell in sechs verschiedene Klassen von Leguminosenanteilen einzuteilen. Dieses Vorgehen führt nicht zu einer wesentlichen Zunahme der Ungenauigkeit für die beiden Klassen mit den geringsten Leguminosenanteilen (unter 15%), welche den grössten Teil der Naturwiesen abdecken. Bei den Klassen mit 30 bis 75% Leguminosen könnte die Ungenauigkeit der jährlich fixierten N-Menge jedoch mehr als 100 kg N ha<sup>-1</sup> betragen.

Das Modell für Körnerleguminosen basiert auf sechs Eingabeparametern und ermöglicht eine fundierte Schätzung der *SNF* über die N-Aufnahme der ober- und unterirdischen Pflanzenteile der Kultur. Die N-Aufnahme kann anhand des vom Landwirt ermittelten Ernteertrags geschätzt werden. Ein wichtiger Parameter ist der N-Ernteindex. Er könnte in Zukunft aus der schweizerischen Sortenprüfung abgeleitet werden und würde auf diese Weise eine sortenspezifische Schätzung der *SNF* erlauben. Für die anderen Parameter, welche kulturspezifisch sind, werden Standardwerte aufgrund der Literatur vorgeschlagen. Die Genauigkeit der Schätzung der fixierten N-Menge hängt stark von der Bestimmung des Ernteertrags ab.

Für Zwischenkulturen sind in der Literatur nur wenige Daten zur *SNF* verfügbar. Für Kleeegrasmischungen können Werte, die auf der schweizerischen Literatur basieren, genommen werden. Allerdings sind die Ergebnisse aufgrund der groben Schätzung des Ertrags und des Leguminosenanteils eher ungenau. Bei Leguminosen-Reinbeständen und Leguminosen-Nichtleguminosen-Mischungen reicht die vorhandene Literatur nicht aus, um eine zuverlässige Schätzmethode vorzuschlagen.

## Résumé

Les bilans import-export de l'azote (N) au niveau de l'entreprise prennent en compte l'ensemble des intrants en azote d'une entreprise, ainsi que les exports de celui-ci sous forme de produits agricoles. Dans plusieurs méthodes de calcul de ce bilan d'azote, les intrants par fixation biologique de l'azote (*BNF*) ne sont toutefois pas inclus en raison des difficultés à quantifier ces flux. Dans les méthodes qui prennent en compte la *BNF*, celle-ci est généralement estimée par des valeurs standards ou par équation empirique simple. Au niveau des entreprises agricoles, la fixation symbiotique de l'azote (*SNF*) contribue généralement de loin le plus à la *BNF*, car par hectare de terres agricoles, seules de petites quantités d'azote sont fixées par fixation asymbiotique, l'autre voie de la *BNF*. La présente étude visait à développer les bases d'une méthode permettant d'estimer la quantité d'azote entrant annuellement dans une entreprise agricole par le biais de la *SNF*, afin que cet intrant puisse être inclus dans le calcul du bilan import-export de l'azote des entreprises agricoles suisses. La méthode considère les prairies permanentes et temporaires, les cultures protéagineuses et les dérobées, et prend en compte la paucité des données disponibles sur les entreprises agricoles.

Deux modèles empiriques pour estimer la quantité d'azote fixée par *SNF* ont été sélectionnés à la suite d'une analyse de littérature, l'un pour les prairies et pâturages, et l'autre pour les cultures protéagineuses annuelles. Dans les deux modèles, la quantité d'azote dans les produits récoltés, qui est liée au rendement de toute culture donnée, est utilisée comme un paramètre d'entrée important. Les modèles sélectionnés ont été adaptés pour mieux représenter les systèmes de production suisses et afin d'utiliser les données disponibles sur les entreprises agricoles.

Le modèle pour les prairies et pâturages est basé sur neuf paramètres d'entrée. Les estimations de la quantité d'azote fixée par *SNF* obtenues avec ce modèle concordent bien avec les résultats d'essais effectués en Suisse. Les estimations sont pourtant sujettes à une grande incertitude en raison de la difficulté de spécifier avec précision les paramètres d'entrée pour les conditions et la gestion spécifiques de l'entreprise. Pour cinq paramètres d'entrée, des valeurs standard basées sur une étude bibliographique approfondie sont proposées, soit parce que l'imprécision causée par l'utilisation de ces valeurs standard est raisonnablement faible, soit parce qu'aucune alternative réalisable n'a pu être identifiée. Ainsi, ces variables sont considérées comme des constantes dans le modèle et n'ont pas besoin d'être déterminées pour les entreprises individuelles. Trois autres paramètres doivent être spécifiques à l'entreprise et peuvent être approximés à partir des données d'entreprise relatif à l'intensité d'utilisation des herbages (rendements, niveau de fertilisation et teneur en azote dans les légumineuses à la récolte). Ces paramètres peuvent donc être approximés avec une quantité de travail similaire à celle requise pour le bilan de fumure faisant actuellement foi (Suisse-Bilanz). Le dernier paramètre, la part de légumineuses dans la communauté végétale, influence fortement l'estimation de la quantité d'azote fixée par les herbages et sa précision. Celle-ci ne peut actuellement être dérivée ni des données d'entreprise existantes ni par télédétection, et son appréciation sur le terrain est laborieuse. En guise de compromis entre précision d'évaluation et charge de travail, nous suggérons de catégoriser visuellement les herbages en six classes différentes de part de légumineuses. Cette approche ne conduit pas à une augmentation substantielle de l'imprécision pour les deux classes avec parts de légumineuses les plus faibles (moins de 15 %), c'est-à-dire pour les classes incluant la majorité des prairies permanentes. Toutefois, l'imprécision de l'évaluation de la quantité d'azote fixée annuellement peut dépasser 100 kg N ha<sup>-1</sup> pour les herbages comptant entre 30 et 75 % de légumineuses.

Le modèle pour les cultures protéagineuses est basé sur six paramètres d'entrée et permet une estimation solide de la *SNF* en utilisant le prélèvement d'azote de la culture (parties aériennes et souterraines de la plante). Le prélèvement d'azote peut être estimé à partir du rendement en grain de la culture, déterminé par les agriculteurs. Un paramètre important est l'indice de récolte de l'azote. À l'avenir, celui-ci pourrait peut-être être mesuré dans le programme Suisse des tests variétaux, ce qui permettrait une estimation de la *SNF* spécifique à la variété. Pour les autres paramètres, qui sont spécifiques à la culture, des valeurs standards sont proposées sur la base de l'étude de littérature. L'exactitude de l'estimation de la quantité d'azote fixée dépend fortement de la détermination du rendement des cultures.

Pour les cultures dérobées, il n'y a que peu de données disponibles dans la littérature. Des valeurs basées sur la littérature suisse peuvent être adoptées pour les mélanges graminées-légumineuses, bien qu'avec une grande imprécision en raison de l'estimation très approximative de la biomasse et de la part de légumineuses. En revanche,

les données bibliographiques sont trop peu nombreuses pour proposer une méthode d'estimation fiable pour les peuplements purs de légumineuses et les mélanges de légumineuses et de non-légumineuses utilisées en tant que dérobées.



## Riassunto

Il bilancio aziendale import-export dell'azoto (N) tiene conto di tutti gli input in un'azienda e delle esportazioni sotto forma di prodotti agricoli. Tuttavia, diversi metodi di calcolo non includono gli input dovuti alla fissazione biologica dell'azoto (BNF) a causa delle difficoltà di quantificazione questi flussi. I metodi che tengono conto della BNF, generalmente adottano valori standard o la stimano tramite semplici equazioni empiriche. Su scala aziendale, la fissazione simbiotica dell'azoto (SNF) generalmente costituisce la maggior parte della BNF, perché per ettaro di terreno agricolo sono fissate solo piccole quantità di azoto mediante fissazione asimbiotica, l'altra via della BNF. Il presente studio è volto a sviluppare le basi di un metodo che permetta la stima della quantità di azoto che entra annualmente in un'azienda agricola attraverso la SNF, in modo che tale input possa essere incluso nel calcolo del bilancio import-export dell'azoto delle aziende agricole svizzere. Il metodo considera prati e pascoli permanenti e temporanei, le colture di proteaginose, le colture intercalari e tiene conto della scarsa disponibilità di dati nelle aziende agricole.

L'analisi della letteratura ha permesso di selezionare due modelli empirici per la stima della quantità di azoto fissata tramite SNF, uno per prati e pascoli e l'altro per le colture proteiche annuali. In entrambi i modelli, la quantità di azoto asportata con la produzione, che è correlata alla resa di un dato raccolto, costituisce un parametro di input fondamentale. I modelli selezionati sono stati adattati per rappresentare meglio i sistemi di produzione svizzeri e per utilizzare i dati disponibili sulle imprese agricole.

Il modello per prati e pascoli si basa su nove parametri di input. Le stime della quantità di azoto fissata tramite SNF ottenute con questo modello concordano con i risultati dei test effettuati in Svizzera. Le stime sono soggette a grande incertezza dovuta alla difficoltà di precisare con accuratezza i parametri di input per specifiche condizioni aziendali e gestionali. Per cinque parametri di input sono proposti valori standard basati sullo studio approfondito della letteratura, sia perché l'imprecisione causata dall'uso di questi valori standard è ragionevolmente bassa, sia perché non è stato possibile trovare un'alternativa. Pertanto, queste variabili sono considerate come costanti del modello e non devono essere determinate per le singole aziende. Altri tre parametri devono essere specifici dell'azienda e possono essere approssimati dai dati relativi all'intensità di utilizzo dei prati e dei pascoli (rese, livello di concimazione e tenore di azoto nelle leguminose al momento della raccolta). Questi parametri possono quindi essere approssimati con una quantità di lavoro simile a quella richiesta per il bilancio degli elementi attualmente in vigore (Suisse-Bilanz). L'ultimo parametro, l'abbondanza relativa di leguminose nella comunità vegetale, influenza fortemente la stima della quantità di azoto fissata dalle superfici erbacee e la sua precisione. Questo non può attualmente essere derivato né dai dati aziendali esistenti né tramite telerilevamento e la sua valutazione sul campo è difficile. Come compromesso tra accuratezza della valutazione e carico di lavoro, suggeriamo di classificare visivamente le praterie in sei diverse classi di abbondanza di leguminose. Questo approccio non comporta un aumento sostanziale dell'imprecisione per le due classi con la quota di leguminose più bassa (inferiore al 15%), cioè per le classi che comprendono la maggior parte dei prati permanenti. Tuttavia, l'imprecisione della valutazione della quantità di azoto fissata annualmente può superare i 100 kg N ha<sup>-1</sup> per prati e pascoli con una quota di leguminose compresa tra il 30% e il 75%.

Il modello per le colture proteiche annuali si basa su sei parametri di input e consente una stima solida della SNF utilizzando l'assorbimento totale di azoto della coltura (organi aerei e sotterranei della pianta). L'assorbimento di azoto può essere stimato dalla resa in granella della coltura, determinata dagli agricoltori. Un parametro importante è l'indice di raccolto dell'azoto. In futuro, se questo parametro fosse misurato nel quadro del Programma svizzero di test delle varietà, consentirebbe una stima della SNF specifica per ogni varietà. Per gli altri parametri, che sono specifici della coltura, sono proposti valori standard sulla base della letteratura. L'accuratezza della stima della quantità di azoto fissata dipende fortemente dalla determinazione della resa del raccolto.

Per le colture intercalari sono disponibili pochi dati in letteratura. Valori ottenuti dalla letteratura svizzera possono essere adottati per le miscele graminacee-leguminose, anche se la stima molto approssimativa della biomassa e della quota di leguminose è fonte di grande imprecisione. Per i popolamenti puri di leguminose e le miscele di leguminose e non leguminose utilizzati come intercalari, i dati bibliografici sono troppo pochi per proporre un metodo di stima affidabile.

# 1 Background and context of the report

## 1.1 Mandate of the Federal Office for Agriculture

The Federal Office for Agriculture (FOAG) aims at developing a tool for the calculation of the farm-gate nutrient balance of Swiss commercial farms. In this context, the FOAG instructed Agroscope to develop the basis for a simple and broadly applicable estimation method of the quantity of nitrogen (N) entering the farms annually through *symbiotic nitrogen fixation (SNF)*, which is to be used as input in the farm-gate nutrient balance. The tool should consider the limited data availability on farms.

## 1.2 Nutrient balance approaches

Approaches to nutrient balancing are differentiated and defined according to the boundaries of the analysis. Three main approaches can be identified: i) farm-gate balance, ii) soil surface balance and iii) system balance (Oenema and Heinen 1999, Watson and Atkinson 1999, Watson *et al.* 2002, Oenema *et al.* 2003).

i) The farm-gate balance considers the inputs and outputs that pass through the farm-gate; less controllable inputs, such as *SNF* or atmospheric deposition, are not always included. It is encouraged by the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR 1995) for calculating the balance at national level and has been used for policy evaluations in Switzerland following the introduction of direct payments in the 1990s (Braun *et al.* 1994, Herzog *et al.* 2007) and as policy measure at farm level, for instance for the now abandoned mineral accounting system (MINAS) in the Netherlands (Oenema and Berentsen 2005).

ii) The soil surface balance considers the inputs to the soil and the outputs via crop removals from the field. OECD and Eurostat (2007) and Eurostat (2013) recommend it for the calculation at national level because the data required are often easier to collect. In contrast to the farm-gate balance, this approach cannot only be calculated at global, national, regional or farm level but also at field level.

The difference between inputs and outputs of the first two approaches yields a surplus or a deficit consisting of total losses to the environment and the changes in soil-nutrient stocks. These types of nutrient balances are indicators for potential current and future nutrient losses from agriculture.

iii) The system balance records not only all nutrient inputs and outputs of the soil surface balance, but also soil-stock changes and losses. This approach also makes it possible to distinguish between different pathways of losses, but it is rarely applied because of the high data requirement.

## 1.3 SNF in the literature on nutrient balance

In the farm-gate approach, as mentioned in section 1.2, *SNF* is not always assessed, arguing that such an input does not pass through the farm-gate (Treacy *et al.* 2008), or due to difficulties of precise estimation (Munters *et al.* 1997, Watson *et al.* 2002). The lack of *SNF* estimation has been recognized as a weak point, among others, of the Netherlands policy tool MINAS (Schröder *et al.* 2003, Oenema and Berentsen 2005).

When *SNF* is included among the N inputs, it is often estimated through empirical relationships or fixed annual values, rarely by direct measurements. Watson *et al.* (2002) found in their review on nutrient budgets in organic farming that among the 20 papers collected (88 farms) only two used direct measurements to assess *SNF*, four did not include any estimation and the remaining used a variety of empirical models. As organic farms rely almost solely on *SNF* as external N input, it is particularly important for them to quantify it. Empirical models are widely used for forage crops, especially for mixed grassland, but in some cases, authors use fixed values according to given classes of legume abundance to simplify the calculations (e.g. Bassanino *et al.* 2007, Dalgaard *et al.* 2012). For pure legume stands or grain legumes, fixed value are often preferred (e.g. Dalgaard *et al.* 2012), but in other cases the fixation is assumed to be the same as the amount of N in the grain content (Haas *et al.* 2006), or is estimated as the difference between the total N uptake and the fertilization rate (Bassanino *et al.* 2007). We found that in the case of the soil surface balance *SNF* is assessed more frequently through empirical models (e.g. Anglade *et al.* 2015, Iannetta *et al.* 2016) than through fixed values as the net N input to soil is more relevant at crop scale.

## 2 Aims, boundaries and approach of the study

The aim of the study was to develop a simple but robust method for the estimation of the quantity of nitrogen entering the farm through biological nitrogen fixation, based on the body of existing literature.

The field of application of the present work is the utilized agricultural area (UAA) of Switzerland. The UAA does not comprise the summering pastures, which are also not included in the Swiss farm balance system (SB), the official whole-farm nutrient balance introduced by the Swiss government (Uebersax and Schuepbach 2004). This study considers all types of grasslands and commonly cultivated crops within the UAA for which the occurrence of symbiotic nitrogen fixation is expected. These are:

- Grasslands:
  - o Permanent grasslands (meadows and pastures)
  - o Temporary grasslands
- Grain legumes grown as pure stands
- Cover crops

In chapter 3 of the present study, we summarize the consulted literature, which consists primarily of recent review papers offering a comprehensive overview of the subject in order to establish a common understanding about the phenomena, the affecting factors and the knowledge underlying *SNF*. Afterwards we review the specific literature sources evaluating different options to quantify the *SNF* in agricultural systems. We focus on the predictive models and their robustness, examining the considered variables and the data requirements.

In chapter 4, we propose two models that could be applied on Swiss farms, one for grassland systems and the second one for grain legumes. Furthermore, adaptations to the models found in the literature were made in order to better represent the specific conditions of Swiss farms. In order to allow an easy and practicable application of the models, we assessed the possibility to adopt standard values for the input variables while preserving reliable estimates of the *SNF*. For the input variables for which no practicable solution was found, we parametrized these values based on the literature. For this purpose, a detailed analysis of the national literature was carried out, and complemented with international sources, if necessary and feasible. The most suitable values to guarantee the reliability of the parameters to be used in order to assure the highest possible accuracy of the estimates are then determined. The variables to be measured directly on farms are identified and methods of determination are proposed. The resulting method is therefore based on models, their parameterization and methods for the evaluation of variables to be determined in the field. These models are then evaluated against their sensitivity and the potential sources of errors to determine their level of accuracy in the quantification of the fixed nitrogen.

### 3 Literature review

#### 3.1 Plant nitrogen allocation and sources

As a basis for *SNF*, it is necessary to understand how plants allocate nitrogen into different plant parts (Fig. 1). Total plant nitrogen can be split up in *above-ground nitrogen (AGN)* and *below-ground nitrogen (BGN)*. *AGN* represents the total amount of nitrogen in the aerial parts of the plants at a given point in time. *AGN* can be further split into *harvested nitrogen ( $N_y$ )*, which is exported from the field, and the *residual above-ground nitrogen, which remains on the field after harvesting and which is represented mainly by stubbles or stubbles and straw ( $N_{stubble}$ )*. The ratio between  $N_y$  and *AGN* is called the *nitrogen harvest index (NHI)* and varies according to species, cultivars and agricultural management, as well as climatic and soil conditions (Fageria 2014). The *NHI* is an important parameter for variety selection in the breeding process as it gives information about the efficiency of a given cultivar to translocate nitrogen to the grains from other tissue. The higher the efficiency and consequently the *NHI*, the higher is the difference between  $N_y$  and  $N_{stubble}$ .

Below-ground nitrogen is defined as the *amount of nitrogen contained in the root system ( $N_{root}$ )* and the *nitrogen derived from rhizodeposition ( $N_{dfr}$ )*, which is still plant-derived nitrogen but is not anymore contained within a well-defined plant structure. Many pathways for  $N_{dfr}$  have been identified: senescence, death and decay of roots and nodules; exudation of soluble compounds; sloughing-off of root border cells, and secretion of mucilage (Fustec *et al.* 2010).  $N_{dfr}$  has to be taken into account for the estimation of the *amount of symbiotically fixed nitrogen ( $N_{fix}$ )* (Carlsson and Huss-Danell 2003, Unkovich *et al.* 2010) because it can exceed 80% of the *BGN* (Høgh-Jensen and Schjoerring 2001).

There are essentially two sources of nitrogen that leguminous plants can draw on: from the soil solution and from the atmosphere through biological fixation. Total nitrogen contained in leguminous plants can therefore be subdivided according to the source from which it was derived (Fig. 1).

At harvest time of both forage and grain legumes, a part of  $N_{fix}$  is removed from the field, letting behind the  $N_{fix}$  accumulated in stubbles, roots, and depending on the system straw. At plot scale, the difference between  $N_{fix}$  and  $N_y$  could be close to zero or even negative for grain legumes, which generally rely on a smaller proportion of *nitrogen derived from the atmosphere ( $PN_{dfa}$ )* than forage legumes, even more so if the straw is removed from the field (Cuttle *et al.* 2003). At farm scale, it is important to distinguish if the harvested product is reused within the farm (e.g. straw) or if it is exported (e.g. grains sold on the market).

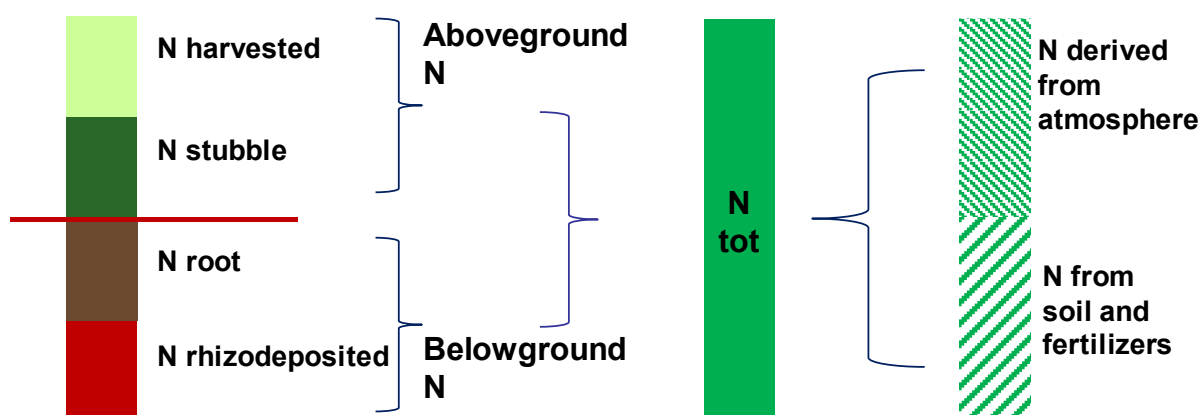


Figure 1. Allocation and sources of nitrogen at the plant level.  $N_{tot}$  is the N uptake; the left side shows the allocation structure above- and below-ground, the right side shows the sources. The figure has only a qualitative purpose as the partitioning varies according to plant species and cultivars and responds to soil conditions and crop management.

## 3.2 Biological nitrogen fixation

*Biological nitrogen fixation (BNF)* is the reduction of atmospheric molecular dinitrogen ( $N_2$ ) to ammonia ( $NH_3$ ) catalysed by the anaerobic enzyme nitrogenase. This is the first step of the N cycle in natural and agricultural systems. The process is only operated by bacteria and archaea and does not occur in eukaryotes. *Symbiotic nitrogen fixation (SNF)* is a particular form of biological nitrogen fixation that includes a mutualistic bond where plants provide a niche and fixed carbon to bacteria in exchange for fixed nitrogen. Moreover, the main enzyme, nitrogenase, used by the microorganisms to fix  $N_2$  is getting irreversibly inhibited by oxygen ( $O_2$ ; Oelze 2000). A very important function of the root nodules produced in the plant-bacteria symbiosis is to protect the nitrogenase from oxygen. In agricultural systems, this symbiosis is restricted mainly to legumes (Mus *et al.* 2016). In legumes, *SNF* ranges from 59% to 93% of the plant nitrogen uptake (Anglade *et al.* 2015), according to species, cultivars, management and climatic conditions. Commonly observed quantities of  $N_{fix}$  through *SNF* in agriculturally used areas containing legumes vary from 30 to 250 kg ha<sup>-1</sup> (Unkovich *et al.* 2008), with quantities above 500 kg ha<sup>-1</sup> being possible (Peoples *et al.* 2019). When legumes are present in the plant community, *SNF* represent by far the major source of atmospheric  $N_2$  to soils (Herridge *et al.* 2008).

*BNF* is also performed by free-living and plant-associated (but not symbiotic) microorganisms. Studies that quantified *asymbiotic nitrogen fixation (ANF)* in agriculturally used areas are scarce. Herridge *et al.* (2008) estimated the magnitude of *ANF* from free-living and associative microorganisms in dryland agriculture to less than 5 kg N ha<sup>-1</sup>. They also mention that the reliability of the evaluation is poor due to scarcity of data. In their literature review, Reed *et al.* (2011) indicated a mean *ANF* rate of 4.7 kg N ha<sup>-1</sup> for temperate grasslands, with a range of 0.1-21 kg N ha<sup>-1</sup>. However, the highest value considered by these authors originates from extrapolation of a laboratory incubation experiment with a soil mixed with ashes to simulate prairie burning (Eisele *et al.* 1989), and is therefore absolutely not representative of conditions found in Switzerland. For conditions much closer to those found in Switzerland (grassland either fertilized and harvested multiple times per year or not fertilized and harvested once a year; Lower Saxony, Germany), Keuter *et al.* (2014) measured a rate of *ANF* of 2.7 kg ha<sup>-1</sup>. Thus, the order of magnitude of *ANF* is hundred times smaller than the one of *SNF* from legume crops or grasslands with a fairly large proportion of legumes (Fig. 2 and 8). Nevertheless, *ANF* is not restricted to legume-containing plant communities and thus, at the farm scale, it represents a background N input of very roughly 100 kg N per year for a 30 ha farm. On the other hand, this background N input also occurs in natural habitats and is more than offset by  $N_2$  lost in the soil-microbe-mediated process of denitrification (Blume *et al.* 2010). Some hypotheses for influencing *ANF* in agricultural soils has been discussed in the literature (Roper and Gupta 2016), but data are too scarce for quantification of the potential effects of agricultural management on *ANF*. Therefore, the current state of knowledge does not allow us to propose any model to estimate *ANF* under different agricultural management practices.

### 3.3 Factors affecting symbiotic nitrogen fixation

Factors that directly influence legume yield (e.g. water and nutrient availability, temperature, incidence of diseases and pests) tend to be the main determinants of  $N_{fix}$  (Lüscher *et al.* 2011), because *SNF* is strongly affected by the N demand of the legumes (Hartwig 1998). Further effects of environmental factors on nodulation and nodule activity have been reviewed by e.g. Liu *et al.* 2011. Succinctly:

- Temperature
  - o Control of nodulation, nodule establishment, and nitrogenase activity
- Soil water
  - o Control of nodule establishment and nodule activity
  - o Water deficit inhibits *SNF*
  - o Water-logging can seriously reduce *SNF* through depression of the establishment and activity of nodules, as well as reduced gas permeability of the soil
- Soil mineral nitrogen in the rhizosphere
  - o Control on nodulation, nodule establishment, and nitrogenase activity
- Inhibition of *SNF* with increasing soil mineral N content. A “starter N effect” has been reported (*i.e.* a small amount of available N in the soil stimulating nodule establishment and *SNF*), but is still controversial. Carbon demand for fixation
  - o Photosynthate partitioned to roots supports nodule growth, provides energy for N fixation, maintains a functional population of rhizobia, and allows the synthesis of amino compounds produced from N fixation.
- Seasonal regulation of *SNF*
  - o Maximum between early flowering and early seed filling
  - o Severe decrease up to cessation after the peak due to nodule senescence

Agricultural management also influences the fixation process. Reviews of these effects can be found in e.g. Cuttle *et al.* (2003) and Peoples *et al.* (2012). Very briefly:

- Proportion of legumes in the plant community:
  - o All agricultural practices modifying the proportion of legumes in multi-species plant communities, mainly grasslands, influence  $N_{fix}$  by influencing legume biomass production. This ranges from the choice of mixtures for the establishment of temporary grasslands to the type and frequency of grassland utilisation and include fertilisation as well as the species of grazing livestock.
  - o Legume proportion also influences  $PN_{dfa}$  by influencing soil N availability to the legume plants (Nyfeler *et al.* 2011).
- Crop rotation and soil tillage:
  - o The position of the legume crops in the crop rotation, the diversity of crops within the rotation as well as tillage may affect *SNF*.
- Nitrogen supply to the crop:
  - o Nitrogen fertilization reduces both  $PN_{dfa}$  and the proportion of legumes in mixed plant communities (Nyfeler *et al.* 2011).
  - o Nitrogen supply to the crop is also influenced by the removal or returning of plant biomass/residues (for instance straw) to the soil. Mulching instead of cutting and harvesting has for instance been found to reduce *SNF* by 30% (Helmert *et al.* 2003)
- Supply of other nutrients to the crop:
  - o Nutrient deficiencies restrict  $N_{fix}$  by hindering plant growth. In the Swiss DOK experiment, for instance, the reduction in  $N_{fix}$  observed under deficient phosphorus and potassium supply was explained by a reduction in clover biomass production rather than by a reduction in  $PN_{dfa}$  (Oberson *et al.* 2013).
  - o Phosphorus deficiency might inhibit nodulation and nitrogenase activity.
- Rhizobial inoculation:
  - o Proper inoculation of legumes with specific rhizobia increases *SNF* where compatible rhizobia are missing in the soil.

### 3.4 Quantification of symbiotic nitrogen fixation

*SNF* may be quantified by direct measurement or by estimation models. This section briefly presents the concept behind the different types of models and discusses their applicability.

#### 3.4.1 Direct measurement

There are several techniques to measure  $N_{fix}$ , both in the field and in controlled environments (Goh *et al.* 1978; Sheehy *et al.*, 1987; Carlsson and Huss-Danell, 2008; Herridge *et al.*, 2008; Unkovich *et al.*, 2008). Almost all these techniques involve destructive sampling of plant and/or soil material, except for the acetylene reduction method (Carlsson and Huss-Danell, 2008). The drawback with the latter method is that it can only be performed in a short time frame (minutes or hours) while the fixation rate varies according to the season and phenological development of the crop; so the period of measurement influences the measurement (Carlsson and Huss-Danell 2008).

#### 3.4.2 Estimations by models

Different approaches to estimating *SNF* using models have been described in the literature. Two main reviews (Cuttle *et al.* 2003; Liu *et al.* 2011) cover this variety of approaches, categorize them and discuss what the most suitable uses for the different categories are. The two main types of existing models are empirical models and mechanistic simulation models (Liu *et al.* 2011).

##### 3.4.2.1 Empirical models

Empirical models are based on observational data, typically using regression techniques, and not on equations describing processes such as physiological processes within the plants like mechanistic models. With respect to *SNF*, this type of models often links  $N_{fix}$  to dry matter (DM) or N yield. The yield reflects the conditions of growth and development, which are therefore taken into account, although indirectly. Further factors can be included in the models if sufficient data from corresponding experiments are available. The advantage of such models is that the number of necessary input variables is typically limited (1 to 6 variables). One of the drawbacks is the impossibility of predicting the outcome for combinations of factors that have not been tested experimentally. There are two main approaches, both of them have to be fed with the  $Y_{DM}$  and  $N_{con}$ , but the concepts behind them are slightly different as we will see in the next two paragraphs.

##### *SNF estimation of grass-legumes forage mixtures*

This method is a direct estimate of *SNF* as parameter values can be measured on site. However, this is often not possible and therefore the values of the parameters have to be extracted from literature and consistent datasets are needed in order to achieve a good estimation. As already mentioned, this model does not take environmental factors such as soil characteristics and weather patterns into account. These are considered indirectly through their influence on the final yield and on the relative abundance of legumes in the plant community. The general model to estimate  $N_{fix}$  in the harvested biomass as formalized by Liu *et al.* (2011) is:

$$N_{fix} = Y_{DM} \cdot Leg_{RA} \cdot N_{con} \cdot PN_{dfa} \quad (1)$$

Where:

$Y_{DM}$  = harvested dry matter yield

$Leg_{RA}$  = relative abundance of legumes in the biomass

$N_{con}$  = N content in the legume fraction of the above-ground biomass

$PN_{dfa}$  = proportion of total plant N derived from atmosphere in the legume fraction

**Variants of equation (1)**

The approach of the equation (1) has been used by different authors. Here we report few examples in which the equation has been modified to take the effect of fertilization into account or to estimate the fraction of N that has not been harvested.

The approach of Korsæth and Eltun (2000) represents the decline of  $N_{dfa}$  in response to N fertilization:

$$N_{fix} = Y_{DM} \cdot Leg_{RA} \cdot N_{con} \cdot PN_{dfa(N_{fert})} \quad (2)$$

Where:

$$PN_{dfa(N_{fert})} = PN_{dfa_{max}} - \gamma N_{fert} \quad (3)$$

Where:  $PN_{dfa_{max}}$  is the maximum proportion of fixation

$\gamma$  is a constant (different values for different species are given in the publication)

$N_{fert}$  is the amount of plant-available nitrogen applied in fertilizers (either mineral fertilizer or slurry/farmyard manure)

The authors also consider a denitrification proportion of 7%, citing experiments in comparable conditions (Ryden, 1985; Svensson *et al.*, 1991; Maag, 1995).

Other authors consider that nitrogen not accumulated in the harvested legume biomass (below cutting height or in companion non-fixing species) have to be taken into account in order to achieve a more complete estimation of the total SNF. The equation is modified as follow:

$$N_{fix} = Y_{DM} \cdot Leg_{RA} \cdot N_{con} \cdot PN_{dfa} \cdot BGTNF \quad (4)$$

For instance Høgh-Jensen *et al.* (2004):

$$BGTNF = (1 + P_{root+stubble} + P_{immobile} + P_{transsoil} + P_{transanimal}) \quad (5)$$

Where:

$BGTNF$  = below-ground and transferred nitrogen factor

$P_{root+stubble}$  = fixed nitrogen in root and stubble as proportion of totally fixed AGN at the end of growing season;

$P_{immobile}$  = fixed nitrogen immobilized in organic soil pool as proportion of fixed AGN at the end of growing season;

$P_{transsoil}$  = below-ground transfer of fixed legume nitrogen located in the grasses in mixtures as proportion of total fixed AGN at the end of growing season;

$P_{transanimal}$  = above-ground transfer (by grazing animals) of fixed legume nitrogen located in the grasses in mixtures as proportion of total fixed AGN at the end of growing season.

**SNF estimation of grain legumes**

Similarly, to the previous, this approach has to be fed with available data, but the underlying concept is slightly different. The assumption of this model is that  $N_{fix}$  is strongly related to the legume yield, which is the main source of variability, a fitting model is then disposed and crop yield is the only parameter that has to be recorded in the field (Bolger *et al.* 1995; Goh and Ridgen 1997; Heuwinkel and Locher 2000; Kumar and Goh 2000; Loges *et al.* 2000; Goh *et al.* 2001; Boller *et al.* 2003; Carlsson and Huss-Danell 2003; Anglade *et al.* 2015). The equation can be summarized as follows:

$$N_{fix} = (\alpha_{crop} \cdot Y_{DM}) + \beta_{crop} \quad (6)$$

Where:

$Y_{DM}$  = dry matter yield (dt DM ha<sup>-1</sup>)

$\alpha$  and  $\beta$  = slope and intercept, determined for each crop.

In an extensive review, Anglade *et al.* (2015) analysed more than 120 papers. They selected long-standing and more recent estimates of SNF for some grain legumes and mixed plant communities across a wide range of environments, with different management practices ranging from conventional to organic, but with a maximum fertilization rate of 150 kg N ha<sup>-1</sup>. The species involved in the relationships are alfalfa (*Medicago sativa*), faba bean (*Vicia faba*), lentil (*Lens culinaris*), field pea (*Pisum sativum*), white clover (*Trifolium repens*), red clover (*T. pratense*), *T. subterraneum*,



*T. alexandrinum*, *T. michelianum*, *T. hybridum*, *T. incarnatum*, *T. resupinatum*. They did not find any correlation between  $Y_{DM}$  and  $PN_{dfa}$  (Fig.2a). However, they found a more robust correlation with AGN ( $R^2=0.92$ , Fig. 2c) than with the harvested dry matter ( $R^2=0.63$ , Fig. 2b).

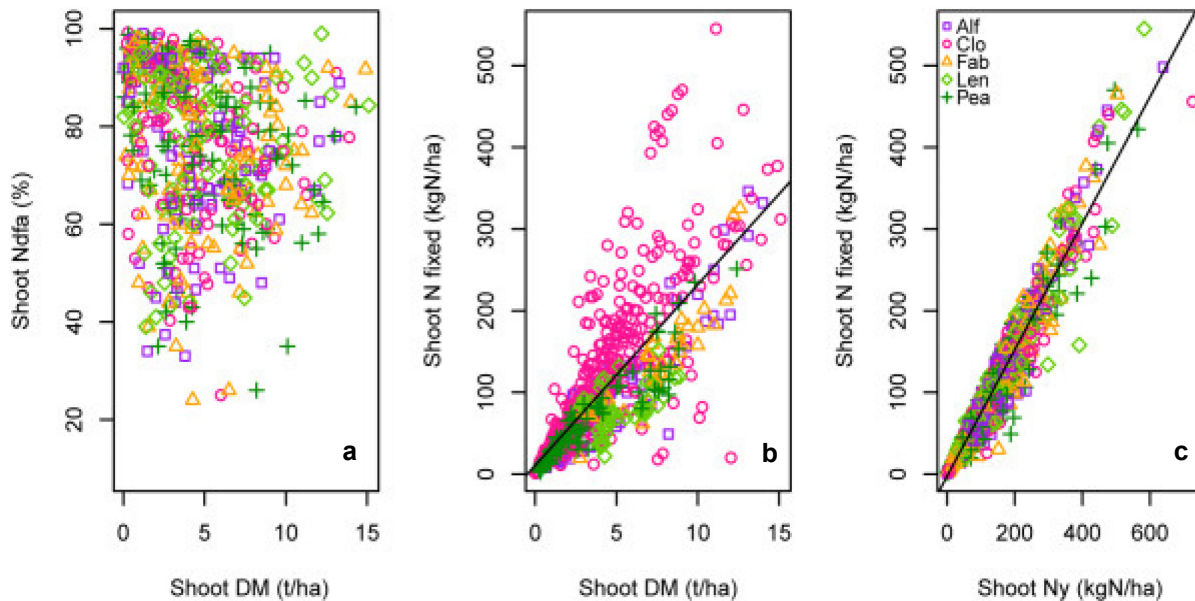


Figure 2. (a): Relationship between the fraction of N in shoot derived from the atmosphere ( $N_{dfa}$ ) and shoot DM produced ( $t\ ha^{-1}$ ) for grain and forage legumes grown in different geographic locations. (b): Relationship between shoot dry matter ( $t\ ha^{-1}$ ) and the amount of  $N_2$  fixed in shoot ( $kg\ N\ ha^{-1}$ ). (c): Relationship between legume AGN ( $kg\ N\ ha^{-1}$ ) and the amount of  $N_2$  fixed in shoot ( $kg\ N\ ha^{-1}$ ). The lines indicate the linear regression among all species (Anglade *et al.* 2015).

Both individual regressions for each crop and general regressions (for all forage crops, all grain legumes and for all crops) have been derived, considering a 95% confidence interval. For all species, except for field pea, the relation with  $N_y$  is more robust than with  $Y_{DM}$  (Tab. 1).

Table 1. Results of linear regressions for different legume crops from Anglade *et al.* (2015) related to dry matter ( $Y_{DM}$ ) and nitrogen yield ( $N_y$ ).  $\alpha$  = slope,  $\alpha\ CI$  = 95% bias corrected and accelerated (BCa) confidence interval of slope,  $\beta$  = intercept,  $\beta\ CI$  = 95% BCa confidence interval of the intercept.

Species	n	$\alpha_{crop}$	$\alpha\ CI$	$\beta_{crop}$	$\beta\ CI$	$R^2$
Alfalfa	123	20.3 $Y_{DM}$	17.6, 22.7	2.49	-5.39, 10.6	0.83
	118	0.81 $N_y$	0.77, 0.86	-13.9	-20.6, -8.67	0.94
Clover	413	25.6 $Y_{DM}$	23.0, 28.2	14.0	7.29, 21.3	0.65
	400	0.78 $N_y$	0.75, 0.82	3.06	-0.56, 7.27	0.94
Forage legumes	536	24.5 $Y_{DM}$	22.4, 26.7	11.0	5.42, 17	0.65
	518	0.79 $N_y$	0.76, 0.82	-0.49	-3.78, 3.29	0.94
Faba bean	39	20.5 $Y_{DM}$	16.8, 25.9	-13.0	-51.7, 10.6	0.79
	82	0.73 $N_y$	0.64, 0.83	5.45	-9.82, 19.3	0.88
Lentil	47	7.46 $Y_{DM}$	3.54, 12.2	39.3	11.7, 61.7	0.20
	74	0.64 $N_y$	0.60, 0.67	3.32	-1.49; 7.14	0.90
Field pea	84	17.6 $Y_{DM}$	15.4, 19.8	6.91	2.54, 11.9	0.86
	186	0.66 $N_y$	0.62, 0.7	4.32	-0.21, 8.53	0.86
Grain legumes	170	17.2 $Y_{DM}$	15.7, 19.3	3.42	-4.83, 9.16	0.79
	342	0.70 $N_y$	0.67, 0.74	1.01	-3.90, 4.96	0.88
All crops	706	22.2 $Y_{DM}$	20.5, 23.9	10.6	5.83, 15.3	0.62
	860	0.77 $N_y$	0.75, 0.8	-2.63	-5.79, 0.43	0.92

The models and relations illustrated above are intended to estimate  $N_{fix}$  in the aerial structures of plants. To determine the total fixed nitrogen by plants (amount of nitrogen fixed in the above-ground structures + amount of nitrogen fixed in the below-ground structures), Anglade *et al.* (2015) introduced a standard factor to take the  $BGN$  into account, defined as the sum of  $N_{root}$  and  $N_{dfr}$ . Considering the results of sixteen studies on forage crops and twenty-one studies on grain legumes, they have set the below-ground N factor at 1.7 (1+0.7) for grasslands and at 1.3 (1+0.3) for grain legumes.

*Glycine max* (soybean) is not considered by Anglade *et al.* (2015), but Salvagiotti *et al.* (2008), with the same approach, proposed a fitting model, in which  $AGN$  is related to  $N_{fix}$  (Fig. 3). They considered 108 published studies including a total of 637 datasets (site–year–treatment combinations) derived from field studies considering  $SNF$  and fertilization over a wide range of soils, climatic conditions, genotypes and management practices.

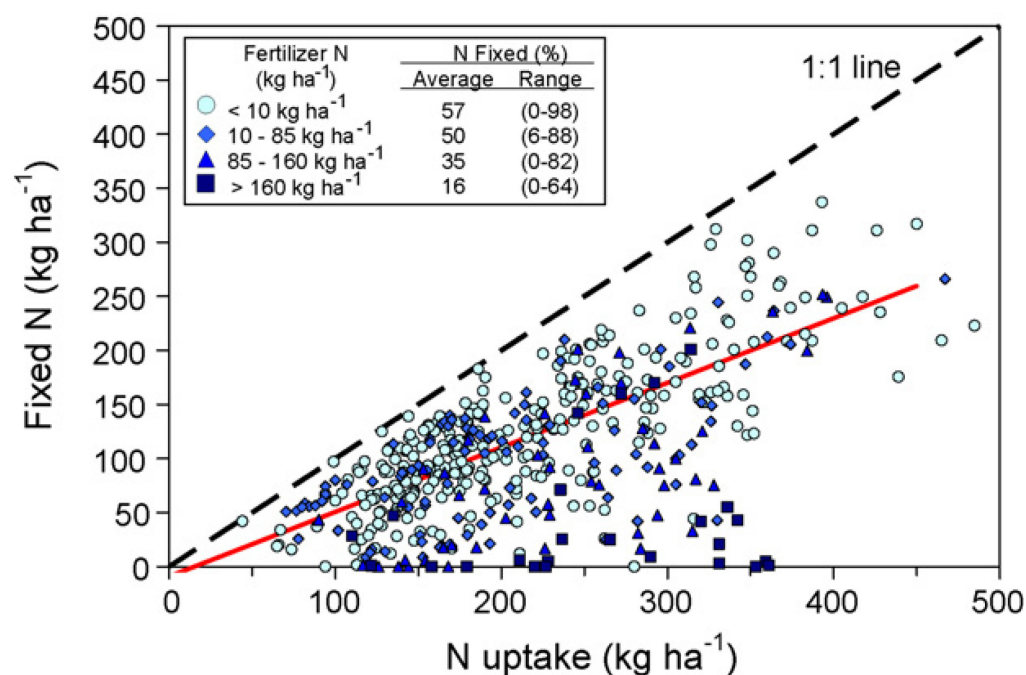


Figure 3. “Relationship between  $N_2$  fixed by soybean and nitrogen uptake in above-ground biomass. The dashed 1:1 line represents values for which all N uptake would be expected to be derived from biological  $N_2$  fixation. Data were divided into four different categories of applied N fertilizer as denoted by the symbols. The solid line is the best linear fit for N fertilizer rates of less than  $10 \text{ kg ha}^{-1}$  ( $y = 0.66x - 19$ ;  $R^2 = 0.59$ ). Values shown refer to  $AGN$ .” (Salvagiotti *et al.* 2008).

Liu *et al.* (2011) discussed that this method (equation 6) is, we cite, “based on statistical correlation and assumes that N fixation has a strong linear relationship to the variables. Compared to equation 1, it is more flexible to use and can be applied to one specific site or multiple sites with different soil types, depending on how the empirical relationship is developed and the sites the data were obtained from. This approach has a higher data requirement compared to the first method (equation 1), and the data should be representative and adequate to guarantee the correlation and determine the parameter values. However, as with the first method (equation 1), these approaches are restricted to specific sites because the equation is not able to represent the interaction between plant and environment mechanistically.”

### 3.4.2.2 Dynamic mechanistic simulation models

From Liu *et al.* (2011): “In more recent simulation models of  $SNF$  in legumes, a popular method to estimate the rate of legume  $SNF$  is a potential or maximum fixation rate modified by the influence of environmental factors. The potential fixation rate is estimated based on either a demand-uptake mechanism or on the dry matter of plant tissues, and is varied with plant growth stages. The environmental factors normally include soil temperature, soil or plant water content, soil mineral N or substrate N concentration in plant tissues and substrate C concentration in the plant. Other factors, such as soil pH, salinity and the supply of other nutrients, have not been yet included in models to date”.

Although the mechanistic approaches are very accurate, they are very demanding in terms of the data needed for calibration. Such data is rarely available for field conditions. However, the information that these models can provide goes well beyond the annual  $N_{fix}$  values. Although these models offer a high-performance, they are not suitable for the purposes of this work.

### 3.5 Conclusions

The processes underlying *BNF* in agricultural ecosystems have been extensively studied. A large array of factors influencing the N fixation of ecosystems has been described and these factors often interact with each other. Correspondingly, measurements of *BNF* in the field have shown huge variations in the amount of nitrogen entering agroecosystems, even for single-species cropping systems (Fig. 3). In agroecosystems containing legumes, two *BNF* pathways occur: asymbiotic biological fixation (*ANF*) and symbiotic nitrogen fixation (*SNF*). For these ecosystems, the latter is quantitatively much larger than the former. Moreover, agricultural management strongly affects the amount of nitrogen entering the production system through *SNF*, while the possibilities of influencing *ANF* are very limited. Consequently, this report focuses on *SNF*. The vast majority of studies on *SNF* focuses on the aerial plant parts. Nevertheless, an important part of the symbiotically fixed nitrogen is allocated to the roots and a sizeable portion of it can be found in the rhizosphere. Data on the below-ground plant N pool (*BGN*) and on rhizodeposition are scarce, which complicates the estimation of the total amount of fixed N entering the ecosystem through *SNF*. In chapter 3 of this report, the main environmental and management factors influencing *SNF* have been described by going deeper into the latter in order to provide an overview of the relationship between *SNF* and agricultural practice.

Different types of models for estimating *SNF* have been proposed in the literature. There is a clear trade-off between the availability of the models to account for a wide array of influence factors and the number of input variables required for the calculation. This work focuses on models using a limited number of input variables being broadly applicable because of the requirement of simplicity and ease of use that are the objective of this work. In any case, the values used for the input variables must be adequate for the models to provide realistic estimations. Mechanistic simulation models can be performant and accurate, but they are not thought to provide simple estimation of  $N_{fix}$ . They better represent the response of plants to specific conditions and are therefore not well adapted to the purpose of this work. A straightforward empirical model (equation 1) has been developed for grasslands, and therefore is more suitable for this type of land use. A second empirical model (equation 6) has been widely used for both forage and grain legumes. In our opinion, it is more suitable to the latter due to its structure based on yield as the only input variable, not considering other factors such as interactions between species. We briefly presented two literature reviews adopting this approach showing robust fits with large datasets. We think that these two empirical models - after some adaptations and simplifications (chapter 4) - are an acceptable compromise between the accuracy of estimations and the ease of use of the method within the frame of a nationwide calculation of farm-gate balance.

## 4 Proposition for the estimation of $N_{fix}$ on Swiss farms

In this chapter we suggest the models including the adaptation and the parameterization that could be applied on Swiss farms.

### 4.1 Symbiotic nitrogen fixation as a proxy for total biological fixation

Because i) *ANF* is typically less than 5 kg N ha<sup>-1</sup>, ii) *SNF* represents approximately 95 to 99% of total biological N<sub>2</sub> fixation on areas planted with legumes or on grasslands (section 3.2), and iii) the inaccuracy of the estimation of the N input through fixation is much larger than 5 kg N ha<sup>-1</sup>, we suggest to use *SNF* as a proxy for total biological fixation for grain legumes and grasslands. We advocate not to calculate any background *ANF* across all agriculturally used areas because this background N input also occurs in natural habitats and is more than offset by N<sub>2</sub> lost in the soil-microbe-mediated process of denitrification (section 3.2) and moreover, robust estimation models are not available.

### 4.2 Grasslands

With respect to the framework conditions for modelling *SNF*, grasslands and grain legume crops differ in a number of ways:

- Grasslands are almost exclusively multi-species communities comprising N fixing as well as non-fixing species.
- The relative abundance of legumes varies widely among grasslands and is known to affect *SNF* (Nyfeler *et al.*, 2011).
- Grassland yield is hardly ever measured on farms.
- The N content of the harvested biomass in grasslands depends, inter alia, on the frequency of defoliation (management intensity).
- N fertilization of grasslands ranges from 0 to 1.2 kg N dt<sup>-1</sup> of harvested DM, and the rate of N fertilization affects *SNF*

Given these particularities, we propose to adopt the empirical model presented in equations (1) to (5) for the permanent as well as for the temporary grasslands, and to consider the effects of N fertilization and of the relative abundance of legumes in the swards on the proportion of nitrogen derived from atmosphere:

$$N_{fix} = Y_{DM} \cdot Leg_{RA} \cdot N_{con} \cdot PN_{dfa(N_{fert}, Leg)} \cdot BGTNF \quad (7)$$

With:

$$PN_{dfa(N_{fert}, Leg)} = PN_{dfa_{ON\_1Leg}} - \gamma N_{fert} + \varepsilon(1 - e^{\kappa(Leg_{RA}-1)}) \quad (8)$$

Where:

$Y_{DM}$  = total dry matter yield (dt ha<sup>-1</sup> year<sup>-1</sup>)

$Leg_{RA}$  = relative abundance of legumes in the biomass (dt DM legumes dt<sup>-1</sup> DM total)

$N_{con}$  = nitrogen content in legumes (kg N dt<sup>-1</sup> DM)

$PN_{dfa(N_{fert}, Leg)}$  = proportion of total plant N derived from the atmosphere in the legume fraction (kg N kg<sup>-1</sup> N), as a function of N fertilization and of the proportion of legumes in the biomass

$BGTNF$  = below-ground and transfer nitrogen factor. It accounts for the amount of symbiotically fixed nitrogen not apportioned to the harvested legume biomass that is for the symbiotically fixed nitrogen allocated to root growth, rhizodeposited or transferred to companion, non-legume species.

$PN_{dfa_{ON\_1Leg}}$  =  $PN_{dfa}$  value for unfertilized ( $N_{fert} = 0$ ) pure stands of forage legumes ( $Leg_{RA} = 1$ )

$\gamma$  = constant that determines the linear effect of  $N_{fert}$  on  $PN_{dfa}$

$N_{fert}$  = rate of nitrogen fertilization (kg N<sub>available</sub> ha<sup>-1</sup> year<sup>-1</sup>)

$\varepsilon$  = difference between the maximum value of  $PN_{dfa}$  (i.e.  $PN_{dfa}$  at  $N_{fert} = 0$  and  $Leg_{RA}$  close to 0) and  $PN_{dfa_{ON\_1Leg}}$

$\kappa$  = constant that determines the curvature of the model for  $PN_{dfa}(N_{fert}, Leg)$ , that is the zeroing speed

## 4.2.1 Parameters and parameterization

### 4.2.1.1 Total dry matter yield ( $Y_{DM}$ )

Grassland yield is hardly ever measured on commercial farms. Nevertheless, an estimation of  $Y_{DM}$  is key to estimate the amount of  $N_{fix}$  by grasslands.  $Y_{DM}$  of different grassland categories is currently estimated on farms, as it is required to fill up the Swiss farm balance system (SB). The calculation is primarily based on a standard value for the consumption of roughage by the livestock of the farm. Moreover, the SB distinguishes eight grassland categories (4 management intensities x 2 management types, i.e. mowing and grazing) with a different production potential for each category and along the altitudinal gradient. For a single year, this method is sensitive to a potential increase or decrease of the stock of roughage on the farm, but this difficulty is reduced when multi-annual averages are considered. We are not aware of a better method for approximating grassland yields at the farm scale, except for tedious field measurements. Reliable remote sensing options to estimate biomass yields of the very heterogeneous grasslands of Switzerland are not yet available (Hart *et al.*, 2020). Stumpf *et al.* (2020) used satellite imagery to map grassland use in Switzerland. They allocated grasslands into two types of usage, mowing or grazing, and three levels of management intensity based on satellite imagery. To help allocating the grasslands into the management intensity classes, they calculated a relative biomass index using the difference in NDVI prior and after the defoliation events, which was appropriate for the objectives of their study. Nevertheless, this does not at all represent an estimation of the amount of forage produced per area unit on the different grasslands, and this amount cannot be simply deduced from such a relative index. In the study of Stumpf *et al.* (2020), this is illustrated by the calculated relative biomass differences between mown and grazed grasslands, which were much larger than the real difference in biomass production between mowing and grazing that can be directly measured in the field (e.g. Husse, 2016).

We therefore suggest to make use of the SB method to estimate  $Y_{DM}$  of each grassland category at the farm scale and use these values in equation 7. This approach does not allow estimating  $N_{fix}$  at the plot level, but an average  $N_{fix}$  value at the level of the grassland categories is sufficient for a farm-gate nutrient balance. We advocate calculating  $Y_{DM}$  at the level of each grassland category rather than an average over all grassland categories of the farm, because it is more straightforward to estimate the other variables of equations 7 and 8 ( $Leg_{RA}$ ,  $N_{con}$ ,  $N_{fert}$ ) at the level of the grassland categories than as means over different grassland categories.

### 4.2.1.2 Relative abundance of legumes ( $Leg_{RA}$ )

An estimation of the relative abundance of legumes on the grassland areas of the farm is necessary to any estimation of  $N_{fix}$ . This parameter should ideally be determined in the field because it does not only depend on management, climatic and soil factors, but also on the competition among species forming the plant community, i.e. on complex environment x management x community interactions. A wide range of nutrient management decisions taken by the farmer might influence  $Leg_{RA}$ . Consequently, it widely varies among farms within each grassland category as defined in the SB. Thus, any approximation from averaged empirical data found in the literature would disregard the specific nutrient management at the farm level, which might be conflicting with the objectives of establishing a farm-gate nutrient balance. Moreover, data on  $Leg_{RA}$  on Swiss farms are very scarce. Information about  $Leg_{RA}$  is not required for the SB nor for other farm documents, and consequently, is not currently available for commercial farms. A visual estimation of  $Leg_{RA}$  at the field scale is currently the less time-consuming method available for assessing  $Leg_{RA}$ . Differentiation in very coarse classes of  $Leg_{RA}$  (less than 30%, between 30 and 50%, more than 50% legumes) is possible with almost no training (Peratoner *et al.*, 2018), but working with such classes for the estimation of  $N_{fix}$  would be extremely imprecise. We suggest using six classes of  $Leg_{RA}$  and discuss the advantages and limits of this suggestion in section 4.2.3.

### 4.2.1.3 Nitrogen content of legumes ( $N_{con}$ )

The total nitrogen content in the legumes ( $N_{con}$ ) can vary according to the species, and, most importantly, with the phenological stage of the plant. Average values of  $N_{con}$  (respectively of the crude protein content) at different phenological stages are given in Agroscope (2016) for the three main forage legumes found in temporary as well as permanent grasslands of the foothill and montane altitudinal zones in Switzerland (Table 2). The data available in Agroscope (2016) are widely used as reference values in Switzerland, including for the Swiss guidelines for fertilizer application in grasslands (Huguenin-Elie *et al.*, 2017).

Table 2. Total nitrogen content of the main forage legume species according to their stage of development and to the growth cycle, and mean values averaged over the three species (Agroscope 2016).

Species	Phenological stage Growth cycle	Total nitrogen content (kg N dt <sup>-1</sup> DM)				
		1	2	3	4	5
White clover	First growth	4.6	4.3	4.2	3.9	3.5
	Regrowths	4.1	3.7	3.6	3.5	3.2
Red Clover	First growth	4.0	3.6	3.2	2.6	2.5
	Regrowths	4.1	3.7	3.2	2.9	2.2
Alfalfa	First growth	4.6	4.4	4.0	3.6	3.1
	Regrowths	4.4	3.9	3.3	2.8	2.6
Mean	First growth	4.4	4.1	3.8	3.4	3.0
	Regrowths	4.2	3.8	3.4	3.0	2.7

A comparison of  $N_{con}$  in red clover grown in intensive temporary grasslands at six locations across Europe indicates that the mean  $N_{con}$  in legume plants harvested at a similar phenological stage is not strongly influenced by the soil and climatic conditions of the sites (Fig. 4). Similarly, the effects on  $N_{con}$  of the level of N fertilization and of the relative abundance of legumes in the sward are not significant (Nyfeler *et al.*, 2011) and can be considered to be negligible for roughly estimating  $N_{fix}$  at the farm scale. This could be explained by the ability of the legume species to regulate their SNF according to their N needs (Hartwig *et al.*, 1998). The moderate variability in  $N_{con}$  among sites, fertilization levels and legume relative abundance allows using average reference values rather than farm-specific estimations or measurements. Moreover, farm-specific measurements of  $N_{con}$  would be extremely tedious, because in Switzerland forage legumes are grown in mixtures with other species (both permanent and temporary grasslands). Thus, we suggest to use the reference values given in Agroscope (2016) to approximate  $N_{con}$ .

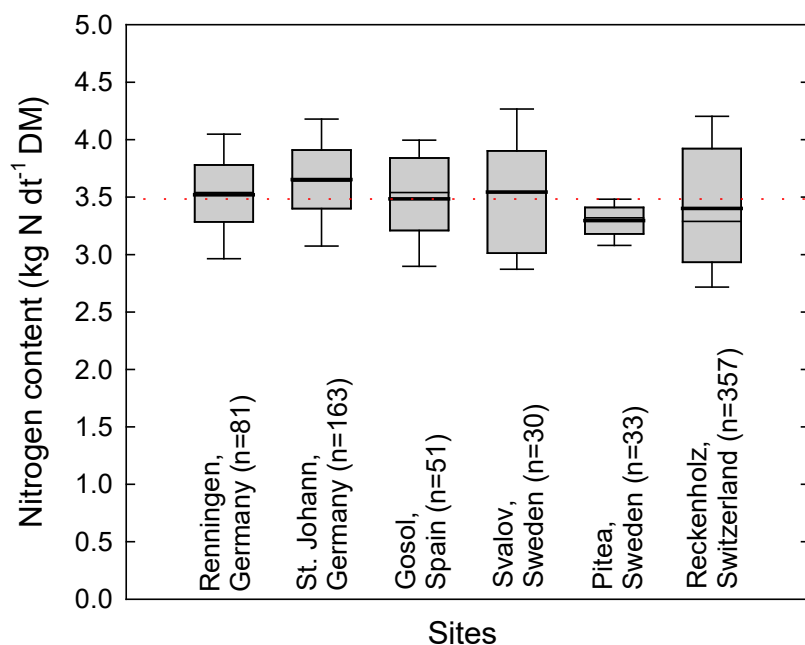


Figure 4. Nitrogen content in red clover (*Trifolium pratense* L.) grown in intensive temporary grasslands at six different locations across Europe and from multiple harvests at each site. The box plots show the mean (bold line), the median (thin line), and the 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup> and 90<sup>th</sup> percentiles. The red dotted line shows the mean  $N_{con}$  across all sites. Data from Kirwan *et al.* (2014).

On a given farm, different legume species could be present on different leys or permanent grasslands. Their respective proportions in the plant community are also expected to be different among grasslands within each farm and to vary from one year to another as well as along the growing season (Hebeisen *et al.*, 1997). A yearly evaluation of the abundance of each species and of the phenological stage of the legumes at each utilization and for each grassland plot seems not feasible within the framework of a countrywide farm-gate nutrient balance, even with

modern proximal sensing approaches currently available (Eriksen *et al.*, 2019). We therefore suggest to approximate  $N_{con}$  using the mean over the three legume species documented in Agroscope (2016) (Table 2). This, together with an estimate of the phenological stage at harvest and of the proportion of biomass originating from the first growth cycle, based on management intensity and management type (Table 3). This approach would provide the advantages of using the same grassland categories for the estimation of  $N_{con}$  as for  $Y_{DM}$ , and of being based on a 1-year time step. In Agroscope (2016),  $N_{con}$  values for the phenological stages 6 and 7 are not available for the legume species. These two late development stages only concern the first growth of extensive pastures and extensive and semi-extensive meadows. We estimated  $N_{con}$  for these stages by linear interpolation from the phenological stages 1 to 5 ( $N_{con}$  estimated at 2.7 and 2.3 kg N dt<sup>-1</sup> DM for stage 6 and 7, respectively) to calculate the weighted  $N_{con}$  means for these three grassland categories.

Table 3. Estimation of the phenological stage at harvest, of the proportion of biomass originating from the first growth cycle, and of the corresponding weighted  $N_{con}$  mean (Agroscope, 2016) for each of the eight grassland categories defined by management intensity and management type according to PRIF (2017).

	Phenological stage		Mean $N_{con}$		Yield proportion 1 <sup>st</sup> growth	Weighted mean $N_{con}$
	1 <sup>st</sup> growth	regrowths	1 <sup>st</sup> growth	regrowths		
<i>Meadows:</i>						
intensive	3	3	3.8	3.4	0.29	3.5
semi-intensive	4	4	3.4	3.0	0.42	3.2
semi-extensive	6	4	2.7	3.0	0.66	2.8
extensive	7	4	2.3	3.0	0.91	2.4
<i>Pastures:</i>						
intensive	2	2	4.1	3.8	0.23	3.8
semi-intensive	3	3	3.8	3.4	0.36	3.5
semi-extensive	5	4	3.0	3.0	0.52	3.0
extensive	6	4	2.7	3.0	0.77	2.8

#### 4.2.1.4 Proportion of total plant nitrogen derived from the atmosphere ( $PN_{dfa}$ )

Measuring  $PN_{dfa}$  requires sophisticated and expensive stable isotope techniques. Therefore, they are no option for  $PN_{dfa}$  determinations within the framework of a countrywide farm-gate nutrient balance. Thus, data available in the literature must be used to estimate  $PN_{dfa}$  in this context. As discussed in section 3.2,  $PN_{dfa}$  is mainly influenced by the level of soil N available to the legume plants (Hartwig *et al.*, 1998). Soil N availability to the legume plants is mainly driven by the rate of N fertilization ( $N_{fert}$ ) and  $Leg_{RA}$  in the plant community (Nyfeler *et al.*, 2011). The effect of  $N_{fert}$  on  $PN_{dfa}$  of forage legumes is large. The body of scientific literature about this effect is listed in table 8. The effect of  $Leg_{RA}$  on  $PN_{dfa}$  has been quantified by Nyfeler *et al.* (2011) and is not negligible. Moreover, farmers can consciously influence these two factors as part of their nutrient management strategy, unlike climatic factors that might have some influence on  $PN_{dfa}$ . We therefore propose to calculate  $PN_{dfa}$  as a function of  $N_{fert}$  and  $Leg_{RA}$  (equation 8).

##### Maximum value of $PN_{dfa}$

$PN_{dfa}$  is considered maximal when only few soil N is available to the legume plants. This is the case when competition for soil mineral N by non-fixing species is maximal, i.e. when  $Leg_{RA}$  is low, and when no (or only few) N fertilization is added. Maximum  $PN_{dfa}$  is used to calculate  $\epsilon$  in equation 8. Measurements of  $PN_{dfa}$  under the conditions mentioned in the first sentence of this paragraph provide values ranging from 0.89 to 0.97 (Table 4). We propose to adopt the mean value of **0.93** of the papers cited in table 4 for the purpose of estimating  $N_{fix}$  within the framework of a countrywide farm-gate nutrient balance.

Table 4. Values of  $PN_{dfa}$  measured in legumes grown in grass-legume-mixture with low relative abundance of legumes and with no or low N fertilization.

Legume species	$N_{fert}$ (kg N ha <sup>-1</sup> year <sup>-1</sup> )	$PN_{dfa}$ (kg N kg <sup>-1</sup> N)	Reference	Note
White clover	0	0.89	Burchill <i>et al.</i> (2014)	2 <sup>nd</sup> year
White clover	0	0.91	Carlsson & Huss-Danell (2003)	75 <sup>th</sup> percentile
White clover	0	0.94	Peoples <i>et al.</i> (2012)	Max of range
White clover	3	0.94	Høgh-Jensen & Schjoerring (1997)	
White clover	32	0.92	Louran <i>et al.</i> (2015)	Weighted mean, 2 <sup>nd</sup> & 3 <sup>rd</sup> year
White and red clover	50	0.94	Nyfeler <i>et al.</i> (2011)	
Red clover	0	0.97	Carlsson and Huss-Danell (2003)	75 <sup>th</sup> percentile
Alfalfa	0	0.90	Peoples <i>et al.</i> (2012)	Max of range
Alfalfa	48	0.93	Louran <i>et al.</i> (2015)	Weigthed mean, 2 <sup>nd</sup> & 3 <sup>rd</sup> year
<b>Mean</b>		<b>0.93</b>		

#### $PN_{dfa}$ value for unfertilized pure stands of forage legumes ( $PN_{dfa\_0N\_1Leg}$ )

The  $PN_{dfa}$  value for unfertilized pure stands of forage legumes ( $PN_{dfa\_0N\_1Leg}$ ) represents the minimal  $PN_{dfa}$  value when no N fertilization is applied.  $PN_{dfa\_0N\_1Leg}$  is the value of  $PN_{dfa}$  when  $Leg_{RA} = 1$  (i.e. 100% legumes in the sward) and when no N fertilization is applied. Thus, it represents  $PN_{dfa}$  without influence of non-fixing plant species in the plant community as well as without influence of added N fertilizer. Studies that measured (or modelled in the case of Fitton *et al.*, 2019)  $PN_{dfa}$  in pure stands of forage legumes with no (or only low) N fertilization are listed in table 5. The range of observed  $PN_{dfa\_0N\_1Leg}$  values is large, with values ranging, for instance, from 0.44 to 0.90 in the multi-site study on alfalfa by Yang *et al.* (2011), and with mean values ranging from 0.60 to 0.86 across the studies found in our literature review (Tab. 5). The range of values reported in the literature review by Carlsson and Huss-Danell (2003) for similar conditions (no or low N fertilization rate and very high legume proportions) is very large as well. This leads to a large uncertainty with respect to  $PN_{dfa\_0N\_1Leg}$  under soil and climatic conditions found in Switzerland. The mean value over the  $PN_{dfa\_0N\_1Leg}$  values reported in table 5 is 0.74, which is close to magnitude of means values presented in the review of Carlsson and Huss-Danell (2003) for all the values considered. Considering an  $PN_{dfa\_0N\_0Leg}$  of 0.93 as described in the previous paragraph and a  $PN_{dfa\_0N\_1Leg}$  of 0.74, the difference ( $\epsilon$  in equation 8) would be 0.19. However, when calculated with the data of Nyfeler *et al.* (2011) for red and white clover,  $\epsilon$  would be 0.30. To our knowledge, this is the only study that measured  $PN_{dfa}$  over a very wide range of legume proportions (from 0 to 1) under the same conditions (one site).

Obviously, the data currently available in the literature allow to determine an order of magnitude for  $PN_{dfa\_0N\_1Leg}$ , and correspondingly  $\epsilon$ , but the accuracy of this parameter of equation 8 has to be considered as low. Nevertheless, because pure stands of forage legumes are only rarely cultivated in Switzerland, the majority of mixtures for temporary grasslands target a legume proportion of less than 0.5 (Suter *et al.*, 2017), and permanent grasslands commonly have a much lower legume proportion, the inaccuracy with respect to  $PN_{dfa\_0N\_1Leg}$  will not translate in a large inaccuracy in  $N_{dfa}$  for the vast majority of grasslands found in Switzerland (Tab. 6). Under these circumstances, we suggest to round up  $\epsilon$  to **0.20**, and correspondingly use  $PN_{dfa\_0N\_1Leg} = \mathbf{0.73}$ .



Table 5. Summary of  $PN_{dfa}$  values found in the literature for forage species grown as pure swards with no or little N fertilization. These values were used to estimate  $PN_{dfa\_0N\_1Leg}$ .

Species	$N_{fert}$ (kg N ha <sup>-1</sup> )	$Leg_{RA}$	$PN_{dfa}$ (kg N kg <sup>-1</sup> N)	Reference
Alfalfa	0	1	0.78	Dhamala <i>et al.</i> (2017a)
	0	1	0.72	Xie <i>et al.</i> (2015)
	0	1	0.67	Yang <i>et al.</i> (2011)
Red clover	0	1	0.79	Boller and Nösberger (1994)
	0	1	0.86	Dhamala <i>et al.</i> (2017a)
White clover	3	1	0.80	Høgh-Jensen and Schjoerring (1997)
	0	1	0.82	Dhamala <i>et al.</i> (2017a)
	0	1	0.81	Jørgensen <i>et al.</i> (1999)
Berseem clover	8	1	0.64	Gianbalvo <i>et al.</i> (2011)
Red and white clover	50	1	0.64	Nyfeler <i>et al.</i> (2011)
	50	1	0.60	Fitton <i>et al.</i> (2019)

Table 6 Effect of the value of  $\epsilon$  on the estimation of  $PN_{dfa}$  for legume proportions of 0.1, 0.2 or 0.5 (at  $N_{fert} = 0$ ).

$\epsilon$	$PN_{dfa\_0N\_100Leg}$	Calculated $PN_{dfa}$		
		$Leg_{RA} = 0.1$	$Leg_{RA} = 0.2$	$Leg_{RA} = 0.5$
0.15	0.78	0.92	0.92	0.90
0.20	0.73	0.92	0.92	0.89
0.25	0.68	0.92	0.91	0.88
0.30	0.63	0.92	0.91	0.87

#### 4.2.1.5 Constant $\kappa$

$\kappa$  represents the curvature of the relationship between  $PN_{dfa}(N_{fert}, Leg)$  and  $Leg_{RA}$  (equation 8), which is the incremental slope of the model for increasing  $Leg_{RA}$ . To our knowledge, the only set of data available for estimating  $\kappa$  is the dataset from Nyfeler *et al.* (2011). The results of Nyfeler *et al.* (2011) and the literature review of Carlsson and Huss-Danell (2003) indicate that  $PN_{dfa}$  is only slightly influenced by  $Leg_{RA}$  in the range of 0 to 50% legumes in the sward ( $Leg_{RA} = 0$  to 0.5). Thus, it only has a marginal effect on the calculated  $PN_{dfa}$  in the usual range of legume proportion in Swiss grasslands. Based on the available data (red and white clover in Nyfeler *et al.*, 2011), we calculated a value of **3.34**.

#### 4.2.1.6 Rate of nitrogen fertilization ( $N_{fert}$ ) and effect on $PN_{dfa}$ ( $\gamma$ )

The rate of nitrogen fertilization ( $N_{fert}$ ) is here used as a proxy for the amount of nitrogen available to the legume plants from sources other than symbiotic fixation. It represents the rate of available N ( $N_{available}$ , defined in annexe 3 of PRIF (2017) as the portion of N available for plants in short or medium term) spread on grasslands with mineral and/or organic fertilizers.  $N_{fert}$  is currently not documented at the plot scale in the SB. Nevertheless, the N requirement of the different grassland categories is calculated in the SB, as well as the difference between the sum of the N requirement of all surfaces of the farm and the sum of plant-available N from the dejections of the farm animals and purchased fertilizers. Thus,  $N_{fert}$  can be roughly estimated at the scale of each grassland category from the SB, assuming fertilizer N is distributed on the farm surfaces proportionately to the calculated N requirements of the different crops and grassland categories. We think that the accuracy of such an approximation of  $N_{fert}$  would be sufficient for the grassland plots of grassland-based milk and meat production systems. This especially because the effect of  $N_{fert}$  on the calculation of SNF with the proposed approach (*i.e.* with an on-farm estimation of  $Leg_{RA}$ ) is quite

weak for  $Leg_{RA} < 50\%$ , i.e. at usual  $Leg_{RA}$  ranges (Fig. 7 and 8). On the other hand, this estimation method for  $N_{fert}$  might be less satisfactory for farms only having a small proportion of grasslands in their utilized agricultural area.

$\gamma$  represents the negative linear effect of  $N_{available}$  on  $PN_{dfa}$ . A negative effect of  $N_{fert}$  on  $PN_{dfa}$  of grassland legumes has been reported in numerous studies (Table 7) and is consistent with the feedback mechanism of soil N availability on SNF described by Hartwig *et al.* (1998). The available data suggest that the effect is linear within a large range of fertilization levels (from 0 to over 400 kg N ha<sup>-1</sup>; Nyfeler *et al.*, 2011 and Fig. 5). The data currently available in the literature does not indicate that the  $N_{fert}$  effect on  $PN_{dfa}$  ( $\gamma$ ) depends on  $Leg_{RA}$ , probably because the response of  $Leg_{RA}$  itself is more sensitive to  $N_{fert}$  than the response of  $PN_{dfa}$  (Oberson *et al.*, 2013). The effect of  $N_{fert}$  was not clearly different in grass-legume-mixtures than in pure legume swards (Table 7). Thus, we suggest to consider  $\gamma$  constant over the whole range of legume proportions. The mean value of  $\gamma$  from our literature review is 0.001, either when calculated as mean values across the mean values of the single studies (Table 7) or as the slope of the linear regression of the combined data points (Fig. 5). Figure 6 shows that  $PN_{dfa}$  calculated with equation 8 and the proposed parameter values plausibly quantify the effects of  $N_{fert}$  and  $Leg_{RA}$  based on measurements performed in an experiment in Switzerland.

Table 7. Summary of the eligible values for  $\gamma$  derived from the available literature with the relative experimental set-up, reference and proposed values as global mean.

Culture conditions	Legume species	$N_{fert}$ range (kg ha <sup>-1</sup> year <sup>-1</sup> )	Slope ( $\gamma$ )	Reference
Mixtures	<i>White clover</i>	0 – 280	0.0010	Burchill <i>et al.</i> (2014)
Mixtures	<i>White clover</i>	3 - 72	0.0008	Høgh-Jensen and Schjoerring (1997)
Mixtures	<i>White clover</i>	0 – 155	0.0004	Oberson <i>et al.</i> (2013)
Mixtures	<i>Red clover</i>	0 – 155	0.0002	Oberson <i>et al.</i> (2013)
Mixtures	<i>White clover</i>	0 – 150	0.0004	Boller and Nösberger (1987)
Mixtures	<i>White clover</i>	100 – 560	0.0007	Zanetti <i>et al.</i> (1996)
Mixtures	<i>White clover</i> , <i>Red clover</i> , <i>T. hybridum</i>	60 – 220	0.0026	Hansen (1995) <sup>1)</sup>
< 50% of legumes	<i>White clover</i>	50 – 450	0.0014	Nyfeler <i>et al.</i> (2014)
< 50% of legumes	<i>Red clover</i>	50 – 450	0.0009	Nyfeler <i>et al.</i> (2014)
Pure stand	<i>White clover</i>	3 – 72	0.0021	Høgh-Jensen and Schjoerring (1997)
Pure stand	<i>Red clover</i>	0 – 240	0.0008	Boller <i>et al.</i> (2003)
Pure stand	<i>White clover</i>	100 – 560	0.0006	Zanetti <i>et al.</i> (1996)
>80% of legumes	<i>White clover</i>	50 – 450	0.0011	Nyfeler <i>et al.</i> (2011)
>80% of legumes	<i>Red clover</i>	50 – 450	0.0010	Nyfeler <i>et al.</i> (2011)
Mean of Mixtures			0.0009	
Mean of pure stands			0.0011	
<b>Proposed value (mean of all)</b>			<b>0.0010</b>	

<sup>1)</sup> As reported in Korsæth and Eltum (2000)

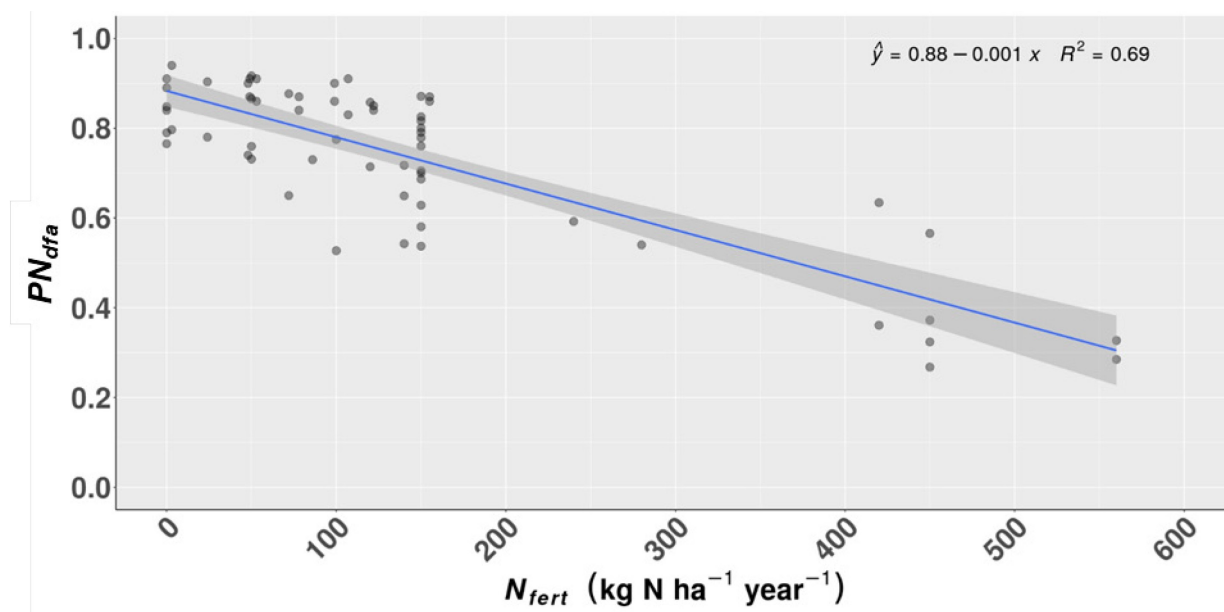


Figure 5. Calculation of  $\gamma$  (slope) by linear regression based the combined data available in the references listed in table 7. The shaded area represents a level of confidence interval of 95% of the prediction model.

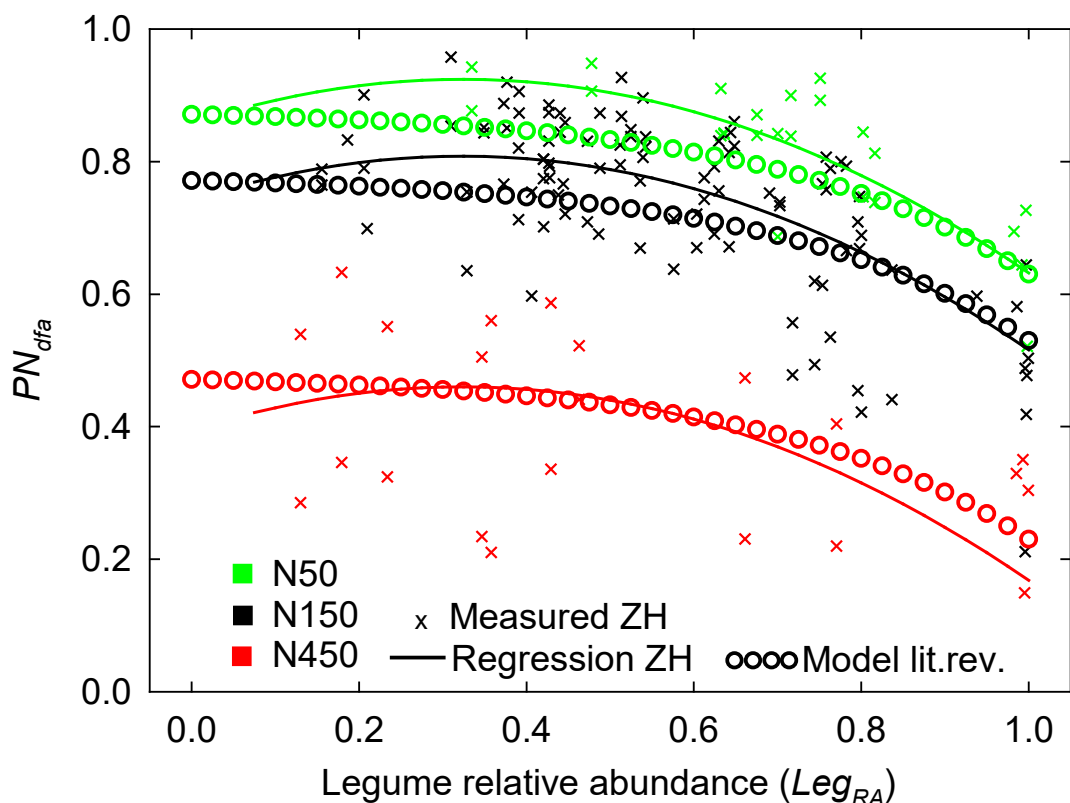


Figure 6. Comparison of the proportion of total plant N derived from fixation in the legume fraction ( $PN_{dfa}$ ) calculated using equation 8 and the parameter values derived from the literature review (Model lit.rev.;  $PN_{dfa\_ON\_1Leg} = 0.73$ ,  $\epsilon = 0.20$ ,  $\kappa = 3.34$ ,  $\gamma = -0.001$ ), with a multiple linear regression (Regression ZH) based on the  $PN_{dfa}$  values measured by Nyfeler et al. (2011) on one site near Zürich (Measured ZH). The figure shows  $PN_{dfa}$  as a function of relative abundance of legumes in the sward ( $Leg_{RA}$ ) and the level of nitrogen fertilization ( $N50 = 50 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ).

#### 4.2.1.7 Below-ground and transfer nitrogen factor (BGTNF)

The below-ground and transfer nitrogen factor represents all nitrogen fixed by the legumes that is not translocated to the harvested legume biomass. At harvest, part of the N once captured by the legume plants is located outside the harvested legume biomass. This is the legume N remaining in the field in stubbles, roots and as rhizodeposition, as well as the  $N_{fix}$  that has been transferred to the companion non-legume species. Part of this N (mainly the N left in the stubbles) is used by the legumes for regrowth following a defoliation event (Høgh-Jensen et al., 2004), and will

therefore be part of the  $N_{fix}$  accounted for at the following harvest. Nevertheless, the N deposited in the rhizosphere as well as the N transferred to companion non-legume species represent inputs of  $N_{fix}$  that are not accounted for when measuring the  $N_{fix}$  in the harvested legume biomass. The vast majority of studies have measured  $N_{fix}$  in the harvested biomass, which therefore represents only part of the total amount of N symbiotically fixed by the legumes. Estimating the amount of  $N_{fix}$  entering the soil-plant system but not being recovered within the harvested legume biomass is necessary to the estimation of total  $N_{fix}$ . Only few estimates of *BGTNF* are available in the literature. In their comprehensive literature review, Anglade *et al.* (2015) considered the data from 16 studies related to below-ground N from forage legumes and reported a mean below-ground factor of 1.7 for forage legumes. A factor of 1.7 means that the size of the below-ground N pool is 70% the size of the above-ground N pool. Anglade *et al.* (2015) defined the below-ground factor as comprising the N associated with roots, nodules and rhizodeposition at crop maturity. Thus, they included the N of the whole root system, which is appropriate for annual crops. For temporary or permanent grasslands, this would overestimate the allocation of N to the root system, because the yearly rate of root turnover is usually less than 1 (Gill and Jackson, 2000; Leifeld *et al.*, 2015). On the other hand, N transfer to companion species seems not to be included in the below-ground factor proposed by Anglade *et al.* (2015). Høgh-Jensen *et al.* (2004) proposed different values for *BGTNF* (subdivided in N in root and stubble, transferred to companion species and immobilized in the soil organic matter) depending on the legume species, the type of management (cutting or grazing), the age of the grassland and the type of soil. The proposed values ranged from 2.45 for grazed, one- to two-year-old grass-white clover swards on clayey soils to 1.45 for mown swards older than two years and on sandy soils. As discussed above with respect to the factor proposed by Anglade *et al.* (2015), they also considered that *BGTNF* might be smaller for multi-year and perennial grasslands than for short-term grasslands. Nevertheless, the data available are scarce and we consider the current state of knowledge not accurate enough to differentiate *BGTNF* for different types of grasslands and environmental conditions. Considering the proportion of temporary and permanent grasslands occurring in Switzerland (17% temporary and 83% permanent grasslands; FOAG, 2018), as well as the fact that temporary grasslands are often cultivated longer than two years in Swiss crop rotations, the smaller *BGTNF* values proposed by Høgh-Jensen *et al.* (2004) are probably more appropriate for the most widespread conditions found in Switzerland than the larger ones. Using  $^{15}\text{N}$  labelling, Hammelehle *et al.* (2018) measured the below-ground N distribution in a red clover-grass mixture in the Swiss DOK experiment (Therwil, BL). They concluded that below-ground N input from red clover grown in mixture with grass represented about 40% of the above-ground N from red clover at any of their three harvest times (after 4, 8 and 19 months of cultivation). This would correspond to a below-ground N factor of 1.4. In another experiment with red clover, Dhamala *et al.* (2017b) also measured a N rhizodeposition by the clover plants corresponding to around 40% of the above-ground clover N (on average of the six mixtures used). A factor of 1.4 (i.e.  $1 + 0.4$ ) is also in the same order of magnitude as the below-ground factor (without transfer) proposed by Høgh-Jensen *et al.* (2004) for grasslands older than two years ( $P_{root+stubble} + P_{immobile} = 1 + 0.25 + 0.19 = 1.44$  on average across clayey and sandy soils). To calculate *BGTNF*, N transfer from legumes to the companion non-legume species has to be added to the measured rhizodeposition (Hammelehle *et al.*, 2018). Values of N transferred from legumes to grasses available in the literature vary from quantities corresponding to 5 to 50% of the amount of above-ground legume N (Louarn *et al.*, 2015; Rasmussen *et al.*, 2019), and the accuracy of the measurement methods is still debated (Peoples *et al.*, 2015). Determining an average N transfer value to be used in a model for the quantification of total  $N_{fix}$  is thus challenging. N transfer seems to be affected by both the donor and the receiver species, with white clover transferring a larger proportion of its N than red clover or alfalfa (Høgh-Jensen and Schjoerring, 2000; Louarn *et al.*, 2015; Rasmussen *et al.*, 2019) and grass species capturing a larger proportion of clover N than forbs (Pirhofer-Walzl *et al.*, 2012; Frankow-Lindberg and Dahlin, 2013; Dhamala *et al.*, 2017b). Estimating the percentage of legume N transferred to the companion species from data available for grass-legume mixtures (Brophy *et al.*, 1987; Dhamala *et al.*, 2017b; Frankow-Lindberg and Dahlin, 2013; Høgh-Jensen and Schjoerring, 2000; Louarn *et al.*, 2015; Oberson *et al.* 2013; Rasmussen *et al.*, 2013; Rasmussen *et al.*, 2019; Schipanski and Drinkwater, 2012; Tzanakakis *et al.*, 2017), we calculated an average N transfer corresponding to 20% of the above-ground legume N across the three species white clover, red clover and alfalfa (arithmetic mean). On the one hand, white clover is arguably more abundant than red clover or alfalfa in Swiss grasslands (larger transfer), but on the other hand, forbs are present in the overwhelming majority of permanent grasslands (lower transfer). Thus, we think that the arithmetic mean across clover species is here a rough but reasonable approximation. N transfer from legumes to the companion species is also influenced by the relative abundance of legumes in the plant community (Brophy *et al.*, 1987; Dhamala *et al.*, 2017b). No N transfer to non-

legumes can, of course, occur in pure legume stands. The results presented by Dhamala *et al.* (2017b) indicate that the percentage of legume N transferred to non-legume species starts declining from legume relative abundances of about 50% onwards. In the absence of more data, we propose to consider the percentage of legume N transferred to companion species constant between almost 0 to 50% legumes (which include the range of  $Leg_{RA}$  in most of the studies and includes the large majority of grasslands in Switzerland), and linearly decreasing from 20% at  $Leg_{RA} = 50\%$  to 0% at  $Leg_{RA} = 100\%$ . To conclude, we suggest to adopt  $BGTNF = 1 + 0.4$  (rhizodeposition) + 0.2 (transfer) = 1.6 for  $Leg_{RA} \leq 0.5$ , and  $BGTNF = 1 + 0.4 + (0.4 \cdot (1 - Leg_{RA}))$  for  $Leg_{RA} > 0.5$  in equation 7 for the approximation of total  $N_{fix}$  in the framework of a countrywide farm-gate nutrient balance. Considerable uncertainty remains with respect to the value of  $BGTNF$ .

#### 4.2.2 Sensitivity of the model

In this section, we test the sensitivity of the model to variation of the different input variables. This shows how the model reacts to an increase or a decrease of the different input variables taken separately. It also shows the effect of incorrectly estimating one of the input variables on the final result of the  $N_{fix}$  estimate. To do so, we incrementally modified each input variable one after another and calculated  $N_{fix}$  at each incremental step with the new set of input variables (Fig. 7). For instance, modifying the input variable  $Y_{DM}$  by -20% alters the calculated  $N_{fix}$  by -20%, while modifying  $\epsilon$  by -20% alters the calculated  $N_{fix}$  by only -3% (Fig. 7). Thus, the calculation of  $N_{fix}$  is much more sensitive to an error in the estimation of  $Y_{DM}$  than to an error in the estimation of  $\epsilon$ . Due to the structure of equation 7, the same relative error for  $Y_{DM}$ ,  $Leg_{RA}$  or  $N_{con}$  leads to the same results. Assuming an effective relative abundance of legumes of 30% ( $Leg_{RA} = 0.3$ ), a 50% overestimation of  $Leg_{RA}$  would result in a 48% overestimation of  $N_{fix}$ , or about 66 kg  $N_{fix}$  ha<sup>-1</sup> for a meadow producing 100 dt DM ha<sup>-1</sup> year<sup>-1</sup>. With visual estimations, errors of more than  $\pm 50\%$  in the estimation  $Leg_{RA}$  are absolutely realistic.

Among the main input variables determining  $PN_{dfa}$ ,  $PN_{dfa\_0N\_1Leg}$  has the greatest influence on the estimation of  $N_{fix}$ , almost comparable to the one of  $Y_{DM}$  or  $N_{con}$ . On the contrary, the variables  $\epsilon$  and  $\gamma$  or  $N_{fert}$  (same effect for  $\gamma$  or  $N_{fert}$ ) have a smaller influence on the estimation of  $N_{fix}$ . With respect to  $N_{fert}$ , it has to be noted that the effect shown in Figure 7 is only the effect of  $N_{fert}$  on  $PN_{dfa}$ . The effect of  $N_{fert}$  on  $Leg_{RA}$  (e.g. Huguenin-Elie *et al.*, 2017) is not considered in the model because the proposed approach assumes that  $Leg_{RA}$  has to be evaluated in the field. In the experiment of Nyfeler *et al.* (2009),  $Leg_{RA}$  was reduced from 24% at  $N_{fert}=50$  to 10% at  $N_{fert}=150$  after three years of cultivation. After this reduction in  $Leg_{RA}$ , the combined effect of  $N_{fert}$  on  $N_{fix}$  estimated with the proposed approach would be of -85 kg  $N_{fix}$  for  $N_{fert}=150$  as compared to  $N_{fert}=50$ , therefore buffering 85% of the supplementary applied  $N_{fert}$ .

As a multiplicative factor,  $BGTNF$  has a strong influence on the estimation of  $N_{fix}$ . Nevertheless, this factor is calculated as 1 plus (+) the proportion of legume N not accumulated in the harvested legume biomass, and the estimation error can only concern this proportion. Thus, the incremental modification of this factor was calculated on 0.6 and not on 1.6. Correspondingly, a 50% overestimation of this factor was calculated as an error of +0.3, resulting in an overestimation of 19% of the calculated  $N_{fix}$ , or about 26 kg  $N_{fix}$  ha<sup>-1</sup> for a meadow producing 100 dt DM ha<sup>-1</sup> year<sup>-1</sup> and having 30% legumes.

The error caused by miss-estimation of legume relative abundance in the field can be substantial and is strongly operator dependent. To mitigate such effect, the legumes abundance could be organized by classes, in this way estimations from different operators would be more homogeneous than continuous estimations.

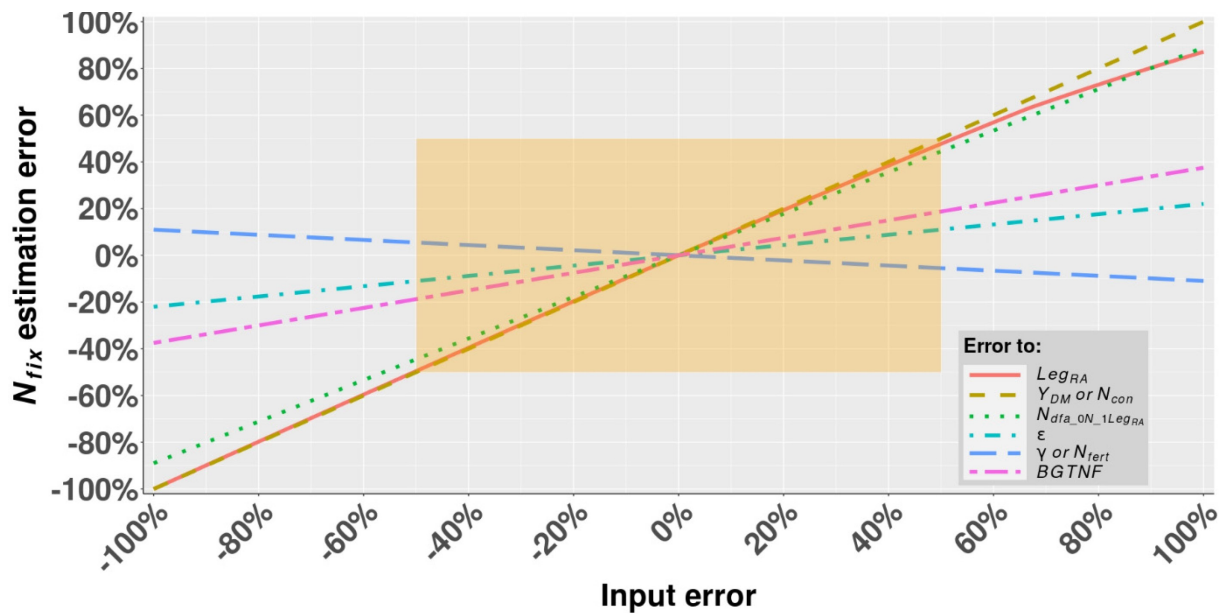


Figure 7. Sensitivity of the model. This figure shows the effect of imprecisions in the quantification of the input variables on the error in estimating  $N_{fix}$ . The calculations have been performed for a meadow with  $Y_{DM}$  of 100 dt ha<sup>-1</sup>, having 30% legumes ( $Leg_{RA} = 0.3$ ), fertilized with 90 kg N ha<sup>-1</sup> and for a phenological stage of 3 at harvest ( $N_{con} = 3.5$  kg N dt<sup>-1</sup> DM). The coloured rectangle represents a range of error of  $\pm 50\%$ .

### 4.2.3 Model output and practical application

#### 4.2.3.1 Output of the model

The amounts of  $N_{fix}$  calculated with the model and the parametrization proposed in section 4.2.1 is presented in figure 8 for a range of conditions. For a grassland producing 100 dt ha<sup>-1</sup> year<sup>-1</sup> and a legume abundance of 30% ( $Leg_{RA} = 0.3$ ), the calculated yearly amount of fixed nitrogen ranges from 133 kg ha<sup>-1</sup> with a fertilization of 120 kg  $N_{available}$  ha<sup>-1</sup> year<sup>-1</sup>, to about 153 kg of fixed N per ha without any fertilization input. These values are higher than the ones reported by Boller *et al.* (2003), Nyfeler *et al.* (2011) and Oberson *et al.* (2013) because they include  $N_{fix}$  in stubbles, roots and as rhizodeposition ( $BGTNF = 1.6$  for  $Leg_{RA} \leq 0.5$ ), while other publications usually report  $N_{fix}$  in the harvested biomass only. It should be noted that legume abundances of more than 50-60% ( $Leg_{RA} = 0.5-0.6$ ) as a mean over the whole growing season are very rare in Swiss grasslands.

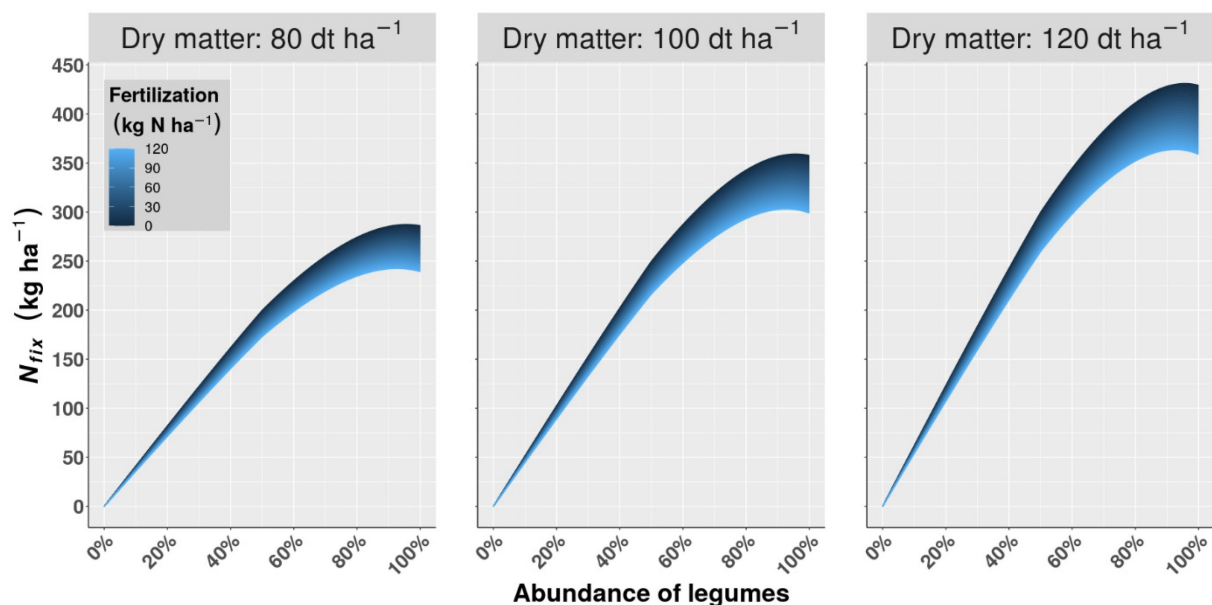


Figure 8. Estimated annual  $N_{fix}$  as affected by  $Y_{DM}$ ,  $Leg_{RA}$  and N fertilization rate at a given  $Leg_{RA}$ .

#### 4.2.3.2 Practical application

Under field conditions, a precise determination of the relative abundance of legumes is very time-consuming and is therefore not realistic within the framework of a countrywide assessment. Realistically,  $Leg_{RA}$  could only be estimated by simple visual assessment. Visual assessments are, of course, not exact and require working with classes of legume relative abundance. The larger the classes of legume relative abundance, the less time-consuming but also the less accurate is the method. Indeed, the inherent inaccuracy due to the difference between the upper and lower limits of each class increases with a decrease in the number of classes. Thus, a trade-off between work-related costs and accuracy has to be found. Class size of 5% legumes corresponds to a mean difference between the lower and upper limit of about 20 kg  $N_{fix}$  per hectare (depending on  $Y_{DM}$ ) while classes of 25% legumes correspond to a difference of more than 100 kg  $N_{fix}$  for  $Leg_{RA}$  between 0 to 0.5 (Fig. 9). With the proposed model, the effect of  $Leg_{RA}$  on  $N_{fix}$  is non-linear, and correspondingly, the division into classes produces a decreasing inaccuracy from the lowest to the highest  $Leg_{RA}$  values (Fig. 9). Thus, we propose a system of six classes that increase progressively in size: 1)  $Leg_{RA} < 0.05$ , 2)  $Leg_{RA} = 0.05 - 0.15$ , 3)  $Leg_{RA} = 0.15 - 0.3$ , 4)  $Leg_{RA} = 0.3 - 0.5$ , 5)  $Leg_{RA} = 0.5 - 0.75$  and 6)  $Leg_{RA} > 0.75$ . This system is similar to those used in phytosociology. The estimated  $N_{fix}$  amount for each class as well as the inaccuracy of the different classes is presented in figure 10 for the example of a grassland producing 100 dt DM  $ha^{-1}$  and fertilized with 100 kg N  $ha^{-1}$ . The class 30-50% of legumes has the highest difference in  $N_{fix}$  between the upper and lower class limit, corresponding to 82 kg  $N_{fix} ha^{-1}$  when  $Y_{DM}=100$  dt DM  $ha^{-1}$  and  $N_{fert}=100$  kg N  $ha^{-1}$ . In proportion to the  $N_{fix}$  amount estimated for this class, the inaccuracy is however less than for the classes <5%, 5-15% and 15-30% legumes (Fig. 10). At first glance, increasing the number of classes might seem a way to reduce this difference. However, it is increasingly difficult to assess visually the relative abundance of a group of plants as the relative abundance of this group increases, and this is the reason why in most systems used in phytosociology, the size of the classes is smaller for the low-abundance-classes than for the high-abundance-classes. The higher the number of classes, the greater the risk of mistaking the class. Moreover, different operators would return less homogeneous and less comparable data using a system with more classes (for example collected in different regions or different years). The higher abundance classes show a lower difference between the upper and lower limit due to the curvature of the estimation model. Thus, increasing the number of classes does not seem justified in the range of  $Leg_{RA} = 0.5$  to 1.0.

The legume relative abundance is known to significantly vary along the vegetation period within each year (Wachendorf *et al.*, 2001). However, to assess this input variable more than once a year is absolutely unrealistic within the framework of a countrywide assessment. For a single assessment per year, the difference in  $Leg_{RA}$  among grasslands is therefore best evaluated when all assessments are performed during the same season. Ideally, the legume relative abundance should be assessed by the same operator in a given region because the effect of the operator on the outcome of a visual assessment is notoriously large.

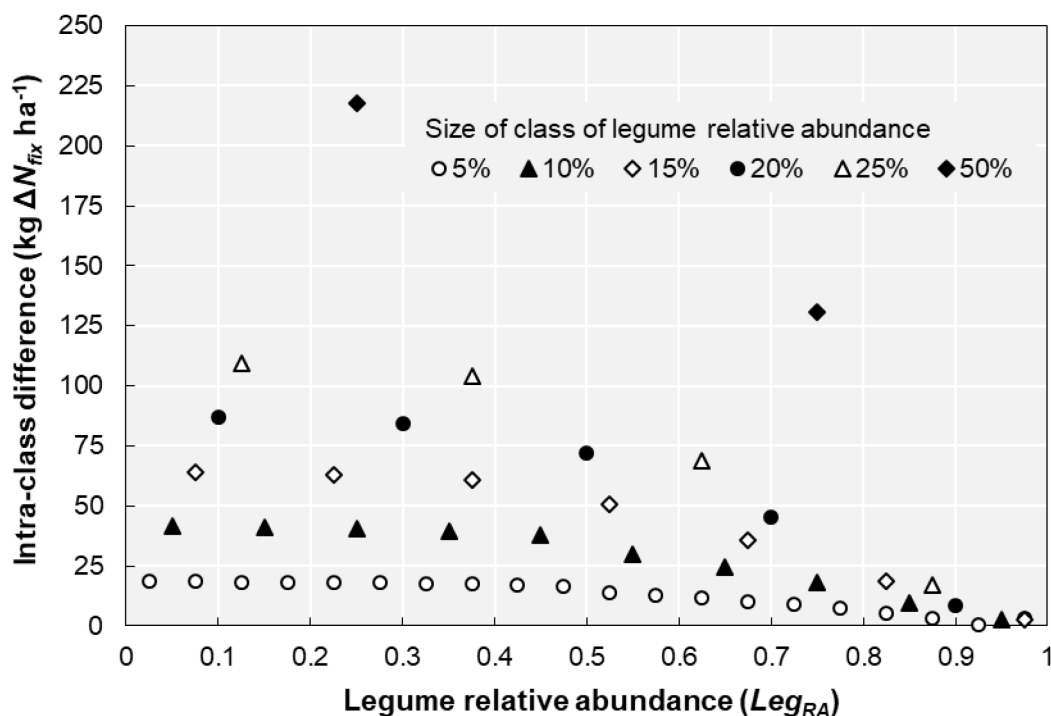


Figure 9. Effect of the size of the classes of legume relative abundance on the inaccuracy of the estimation of annual  $N_{fix}$  ha<sup>-1</sup> within a class. The symbols show the difference in  $N_{fix}$  between the upper and the lower limit of the corresponding class for different class sizes and across the range of possible legume relative abundances. For instance, when using the class  $Leg_{RA}(0-0.25)$ , i.e. a class size of 25% legumes at the lowest range of possible legume relative abundances, the same  $N_{fix}$  would be estimated for grasslands with 0 and with 25% legumes although the difference in  $N_{fix}$  between these two types of grasslands would be of about 110 kg ha<sup>-1</sup> year<sup>-1</sup>. The values have been calculated for a grassland producing 100 dt DM ha<sup>-1</sup> and a fertilization rate of 100 kg N ha<sup>-1</sup> year<sup>-1</sup>.

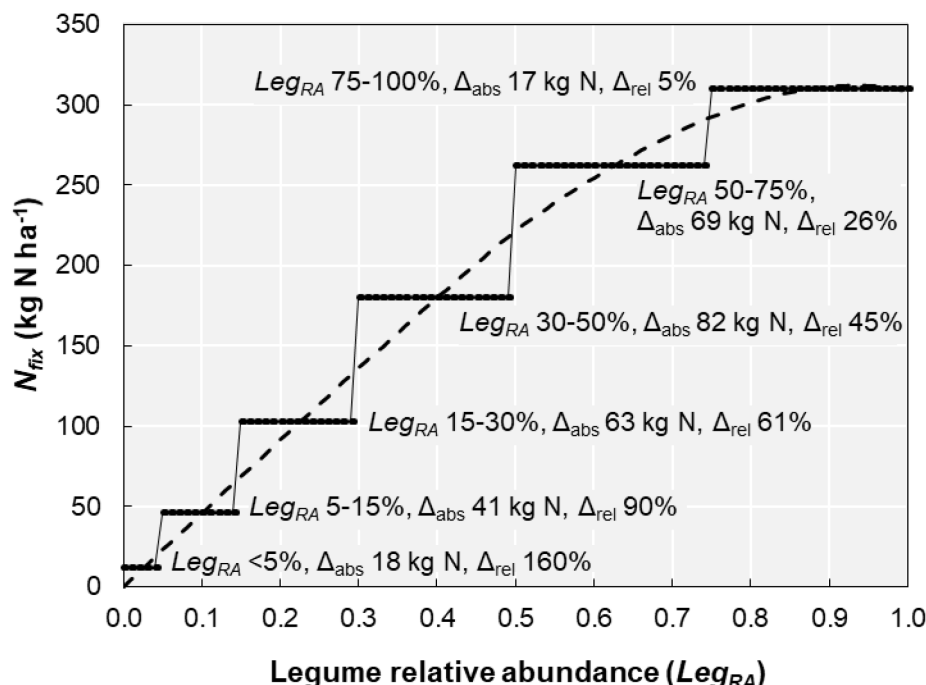


Figure 10. Proposed classes of legume relative abundance and their effect on the estimated annual amount of  $N_{fix}$  ha<sup>-1</sup>. The stair-like curve shows the  $N_{fix}$  amount estimated using the proposed  $Leg_{RA}$  classes for the example of a grassland producing 100 dt DM ha<sup>-1</sup> and a fertilization rate of 100 kg N ha<sup>-1</sup> year<sup>-1</sup>. The absolute as well as the relative difference in  $N_{fix}$  between the upper and the lower limit of each class ( $\Delta_{abs}$ , and respectively  $\Delta_{rel}$ , relative to the estimated  $N_{fix}$  amount of the class) are written in the figure. The dashed line shows the  $N_{fix}$  amount estimated with a continuous increase in  $Leg_{RA}$ .



#### 4.2.4 Conclusions

The proposed model for the estimation of  $N_{fix}$  by grasslands requires nine input variables (equations 7 and 8). We suggest default values for five of them (Tab. 8). Thus, these five input variables can be considered as equation constants that do not need to be specified for the specific farms. For one input variable ( $N_{con}$ ), default values specified for the different grassland categories as defined in the current SB can be used. Two other input variables ( $Y_{DM}$  and  $N_{fert}$ ) can be approximated using data available in the SB. On the contrary,  $Leg_{RA}$  requires a tremendous amount of supplementary work (as compared to the SB) from the farmers or the enforcement authority to be appropriately estimated. As a trade-off between accuracy and workload, we suggest an evaluation system using visual assessments and six classes of legume relative abundances. The proposed model is therefore as follow:

$$N_{fix} = Y_{DM} \cdot Leg_{RA(class)} \cdot N_{con(cat)} \cdot \left( 0.73 - 0.001N_{fert} + 0.2 \left( 1 - e^{3.34(Leg_{RA(class)}-1)} \right) \right) \cdot BGTNF_{(Leg_{RA(class)})}$$

With:

$Leg_{RA(class)}$  = median  $Leg_{RA}$  value between the lower and upper limits of the assessed  $Leg_{RA}$  class

$N_{con(cat)}$  =  $N_{con}$  depending on the grassland category according to table 3

$BGTNF_{(Leg_{RA})}$  = 1.6 for  $Leg_{RA} \leq 0.5$ , respectively 1.8 - 0.4  $Leg_{RA}$  for  $Leg_{RA} > 0.5$

The size of the effect of each input variable on the estimation of  $N_{fix}$  as well as our appraisal of the accuracy of estimation of these variables are summarized in table 8. These two aspects help assessing how critical each input variable is with respect to the risk of inaccurate  $N_{fix}$  estimation. The variables  $Y_{DM}$ ,  $Leg_{RA}$ ,  $N_{con}$  and  $PN_{dfa}$  (mainly determined by  $PN_{dfa\_0N\_1Leg}$  in combination with  $\epsilon$ ) make the greatest contribution to the estimation of  $N_{fix}$ . Among them,  $Leg_{RA}$  is particularly challenging to quantify. It is also crucial for any sensible estimation of  $N_{fix}$ , as well as for any consideration about the effects of farm-specific grassland management on  $N_{fix}$ . To forgo the estimation of  $Leg_{RA}$  on the grassland areas of the farm would indeed result in an extremely inaccurate appraisal of  $N_{fix}$ . Using default values for  $Leg_{RA}$  could easily bring about inaccuracies of  $\pm 100\%$  in its estimation and correspondingly inaccuracies of  $\pm 100\%$  in the estimation of  $N_{fix}$ .  $Y_{DM}$  is affected by a rather important estimation inaccuracy. However, with the proposed method of estimating it based on standard values for the consumption of roughage by the livestock of the farm (SB), it is unlikely to make a very large error, for instance of  $\pm 50\%$ , when estimating  $Y_{DM}$ .  $BGTNF$  has a great effect on the final estimate of  $N_{fix}$  and its accuracy must be considered poor. Unfortunately, the body of literature about this variable is very fragmentary and no alternative other than using a  $Leg_{RA}$ -dependent default value is available at the moment. An estimation of  $N_{fert}$  using the SB can be expected to be quite inaccurate, but  $N_{fert}$  is not one of the main direct contributors to the value of  $N_{fix}$ . The main effect of  $N_{fert}$  on  $N_{fix}$  is through its strong effect on the relative abundance of legumes in the plant community. This effect is thus considered with the input variable  $Leg_{RA}$  and it is therefore essential that  $Leg_{RA}$  is evaluated directly in the field.

Table 8. Summary of the variables of the estimation model and their characteristics.

Input variable	Effect of variable on result	Proposed source	Work required	Accuracy of estimation of variable	Risk for inaccurate $N_{fix}$ estimation	Alternative
$Leg_{RA}$	Very large	On-farm visual estimation	Very substantial	Fairly good <sup>1)</sup> to poor <sup>2)</sup>	Moderate to large depending on accuracy of $Leg_{RA}$ estimation	The use of default values would be extremely inaccurate
$Y_{DM}$	Very large	SB (fodder consumption balance)	Moderate	Quite poor	Quite large	On-farm measurements would be very time-consuming
$BGTNF$	Large	Default value ( $Leg_{RA}$ -dependent)	None	Poor	Large	None
$N_{con}$	Very large	Default values, per grassland category	Little	Fairly good	Fairly small	Laboratory analysis would be time-consuming and expensive
$N_{fert}$	Small	SB	Moderate	Quite poor	Fairly small <sup>3) 4)</sup>	Fertilization plan or report of the real fertilizer application
$PN_{dfa\_0N\_1Leg}$	Very large	Default value	None	Quite poor	Fairly small <sup>4)</sup>	None
$\varepsilon$	Moderate	Default value	None	Quite poor	Fairly small <sup>4)</sup>	None
$\gamma$	Small	Default value	None	Quite poor	Small	None
$\kappa$	Very small	Default value	None	Poor	Small	None

<sup>1)</sup> If performed by a trained professional on all grassland surfaces of the farm.

<sup>2)</sup> If performed by an untrained individual or on a small percentage of the grassland surfaces of the farm.

<sup>3)</sup> Provided that  $Leg_{RA}$  is evaluated on farm.

<sup>4)</sup> Within the most usual range of legume relative abundance (<50%).

### 4.3 Grain legumes

In Switzerland, the importance of grain legumes is increasing and a further increase can be expected in the future, both because of their ability to introduce low-cost nitrogen into crop rotations and because of the possibility of increasing the self-sufficiency level for protein in livestock production chains and human nutrition. The most important grain legumes in Switzerland are field pea, soybean and faba bean (Tab. 9).

Table 9. Evolution of the surfaces (in ha) and the yields (in dt ha<sup>-1</sup>) of grain legumes in Switzerland (agristat 2022).

Crop	Unit	2010	2015	2020
Field pea	ha	3483	4355	3573
	dt ha <sup>-1</sup>	41.7	34.5	28.5
Soybean	ha	1087	1719	2032
	dt ha <sup>-1</sup>	28.3	23.6	25.8
Faba bean	ha	274	556	957
	dt ha <sup>-1</sup>	31.0	26.5	20.2
Mix with cereals	ha	-	409	679
	dt ha <sup>-1</sup>	-	38.9	42.4
Lupin	ha	59	105	210
	dt ha <sup>-1</sup>	32.3	29.5	23.8
Lentils	ha	-	70	135
	dt ha <sup>-1</sup>	-	-	-

In section 3.4.2 we present an empirical model that puts  $N_{fix}$  in relation with  $Y_{DM}$  (equation 6) and we also report that in the review of Anglade *et al.* (2015) were found more robust relations between  $N_{fix}$  and  $AGN$  instead of  $Y_{DM}$ . Anglade *et al.* (2015) also introduced a factor to take  $BGN$  into account. Thus, we propose to adopt the model formalized as follows:

$$N_{fix} = (\alpha_{crop} \cdot Y_{DM} \cdot N_{con} \cdot NHI^{-1} + \beta_{crop}) * BGNF \quad Y_{DM} > 0 \quad (9)$$

Where:

$\alpha_{crop}$  and  $\beta_{crop}$  are the slope and the intercept determined by regressions specifically for each crop

$Y_{DM}$  = harvested grain dry matter yield (dt DM ha<sup>-1</sup>)

$N_{con}$  = nitrogen concentration in grain (kg N dt<sup>-1</sup> DM)

$NHI$  = nitrogen harvest index, defined as the ratio of the harvested N (=  $Y_{DM} * N_{con}$ ) to the above-ground N ( $AGN$ )

$BGNF$  = below-ground nitrogen factor.

This model does not take into account the effect of N fertilizer on  $SNF$  because the recommended N fertilizer application rate is 0 kg N ha<sup>-1</sup> year<sup>-1</sup> for all the grain legume crops in the Swiss guidelines for fertilizer application (PRIF 2017).

#### 4.3.1 Parameterization

Using the estimation model presented in equation 9, only yield ( $Y_{DM}$ : the grain dry matter yield in dt ha<sup>-1</sup>) has to be determined on the farms.

$\alpha_{crop}$  and  $\beta_{crop}$ : are the slope and the intercept of the model, which are determined experimentally or by literature review, as mentioned in chapter 3. The review of Anglade *et al.* (2015) is the most recent and complete work on this topic, which was designed for European conditions (the papers considered in this review are not all European). For these reasons, we propose to use the value issued by this document (Tab. 1) on the basis of crops (species). For soybean, which is not included in the review of Anglade *et al.* (2015), we propose to rely on the value proposed by Salvagiotti *et al.* (2008) in their monographic review for fertilization rates below 10 kg N ha<sup>-1</sup> year<sup>-1</sup> (Fig. 3). Also, in this case the determination was made at the species level.

For *Lupinus* spp. (lupin) we did not find consistent datasets in literature. As Herridge *et al.* (2008) found a similar  $PN_{dfa}$  as for faba bean and put the two crop types in the same category, we propose to adopt  $\alpha_{crop}$  and  $\beta_{crop}$  from faba bean or alternatively to use the parameter for “all grains”.

Grain nitrogen concentrations ( $N_{con}$ ) in grain legumes vary among species, cultivars, sites and years of cultivation. Average values of  $N_{con}$  (respectively of the crude protein content) in grain legumes intended as feedstuff are given in Agroscope (2016) for the most relevant species (Tab. 10).

Table 10. Grain nitrogen content of grain legumes intended as feedstuff (Agroscope 2016).

Crop	$N_{con}$ (kg N dt <sup>-1</sup> )
Faba bean	4.74
White lupin ( <i>Lupinus albus</i> )	5.88
Blue lupin ( <i>Lupinus angustifolium</i> )	5.58
Field pea	3.43
Soybean	6.33

An overview of the variability of  $N_{con}$  give the datasets by Anglade *et al.* (2015) and by Agroscope’s variety test program (Baux *et al.* 2015, Baux *et al.* 2016, Schwaerzel *et al.* 2018, Fig. 11). Data from Switzerland look less variable than those from Anglade *et al.* (2015), but the mean values are in the very same magnitude than those proposed by Agroscope (2016) for faba bean, field pea and soybean. For lentil we could not find large datasets, but as shown in figure 11 its nitrogen content is in the very same range as that of field pea. We propose therefore to adopt the value of field pea. Soybean can be used as feedstuff, prime matter for oil or tofu. Normally the variety used for tofu show higher N contents than others as shown by the results of the variety test program (Fig. 12). We propose to adopt the value of 7.3 kg N dt<sup>-1</sup> DM for the varieties intended for tofu, and 6.33 kg N dt<sup>-1</sup> (Agroscope 2016, Tab. 10) for other purposes. A summary of the proposed value is presented in table 12.

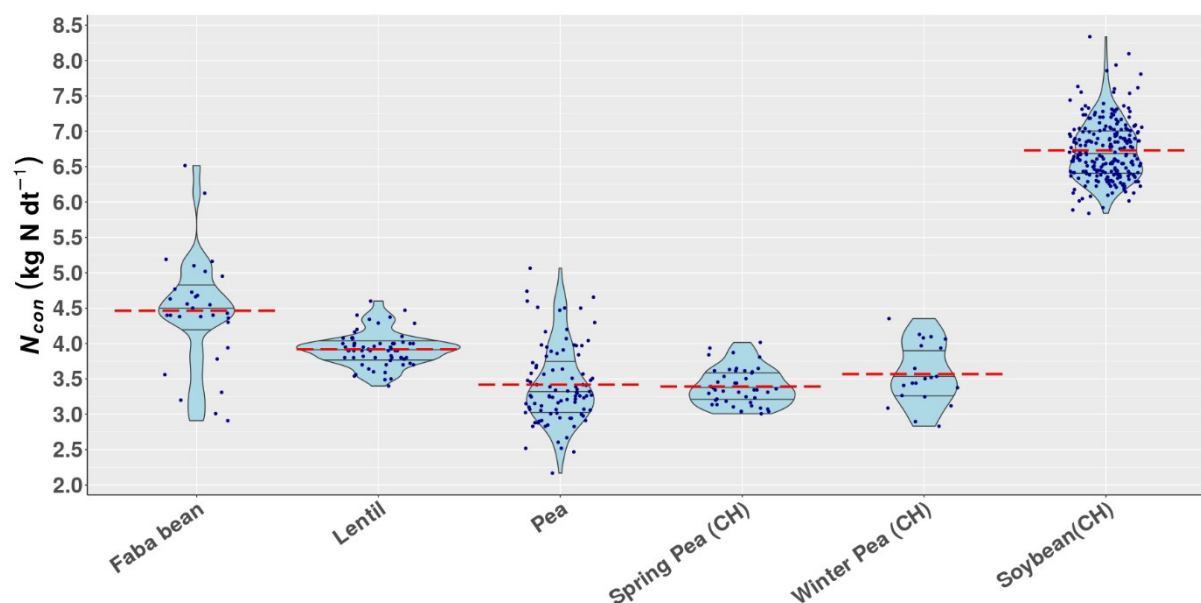


Figure 11. Nitrogen concentration (kg N dt<sup>-1</sup> DM) of grain legume species grown in Switzerland. The violin shapes represent the kernel density: the larger the shape, the higher the density. The black line inside the violin represents the quartile (25%, 50% and 75%). The red dashed line represents the mean. Source: Anglade *et al.* (2015) for faba bean, lentil and field pea, crops followed by (CH) are the dataset from Agroscope’s variety test program (Baux *et al.* 2015, Baux *et al.* 2016, Schwaerzel *et al.* 2017).

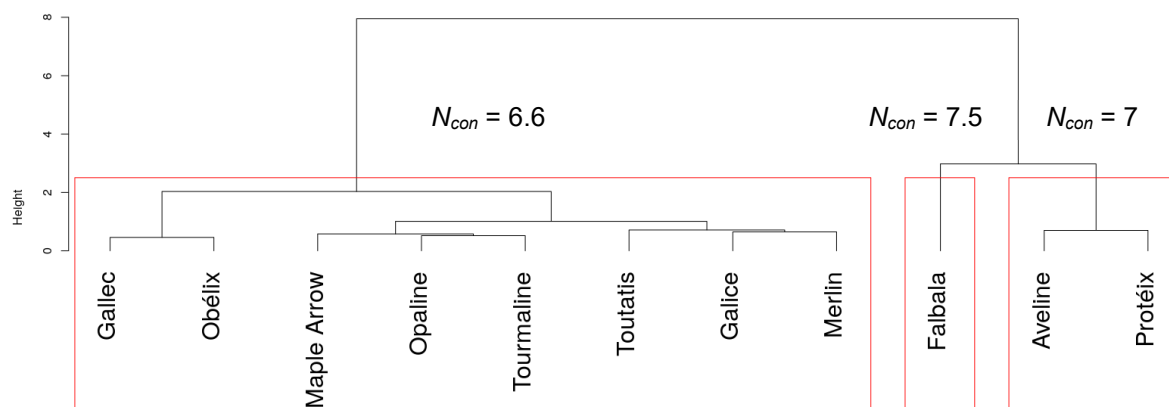


Figure 12. Classification by cluster analysis (Euclidean distance, Ward’s method) of the varieties tested by Agroscope according their  $N_{con}$  (kg N dt<sup>-1</sup> DM). The varieties “Falbala”, “Aveline” and “Protéix” are commonly used in tofu production, others for oil production or as feedstuff.

**NHI:** The ratio between the harvested and the above-ground N is a parameter difficult to obtain as it varies among crop species, soil types and farming practices (Fageria 2014). Unfortunately, the determination of this parameter is not part of routine analysis of Agroscope’s variety test program and we did not find any recent measurements carried out in Switzerland. However, valuable sources for the *NHI* can be found in the international literature: we propose to adopt the values presented by Salvagiotti *et al.* (2008) for soybean and by Anglade *et al.* (2015) for faba bean, lentil and field pea (Tab. 11). As Anglade *et al.* (2015) did not find any significant difference between the *NHI* of the different crops, we propose to adopt the value of 75% for all the grain legumes, including lupin (for which data on *NHI* are scarce) and for soybean, as slight errors in estimating *NHI* will lead to negligible differences in the  $N_{fix}$  estimation (see section 4.3.2).

Table 11. Nitrogen harvest index of the main grain legumes grown in Switzerland. Bold numbers are the median (faba bean, lentil and field pea) and the mean (soybean) values. Ranges below are the interquartile ranges.

Crop	<i>NHI</i>	Source
Faba bean	<b>0.74</b> 0.63 – 0.77	Anglade <i>et al.</i> (2015)
Lentil	<b>0.75</b> 0.63 - 0.78	
Field pea	<b>0.75</b> 0.68 - 0.82	
Soybean	<b>0.73</b> 0.64 - 0.82	Salvagiotti <i>et al.</i> (2008)

As shown in section 3.1.2, it is crucial to adequately quantify the *BGN* and the residual N from not harvested material in order to achieve a good estimation of the total nitrogen fixed by crops. However, due to differences in experimental protocols, root morphology and responses to management, the available data from literature can provide only some indicative values.

Anglade *et al.* (2015) do not treat soybean and Salvagiotti *et al.* (2008) do not attempt to define a value for soybean. However, Rochester *et al.* (1998) reported that 24% of total nitrogen uptake is located in roots and data from Schweiger *et al.* (2012) allow to state an average of 34%. These values are within the same order of magnitude as those considered by Anglade *et al.* (2015) for others legume crops.

As the data available are scarce, we propose to adopt the value of **1.3** (= 1.0+0.3; Tab. 12), proposed in the review of Anglade *et al.* (2015) for the below-ground nitrogen factor (*BGNF*). The lower value of *BGNF* compared to the *BGTNF* that we proposed for grassland systems is due to the fact that in the *BGNF* no transfer to companion crops is considered and *BGN* inputs are smaller due to less root turnover in annual crops compared to frequently cut perennial crops.

Table 12. Summary of the proposed values for the parametrization of equation 9.

	$\alpha_{crop}$	$\beta_{crop}$	$N_{con}$ (kg N dt <sup>-1</sup> DM)	$NHI$ (N <sub>y</sub> AGN <sup>-1</sup> )	$BGNF$
Faba bean	0.73	5.45	4.46	0.75	1.3
Lentil	0.64	3.32	3.43	0.75	1.3
Field pea	0.66	4.32	3.43	0.75	1.3
Soybean	0.66	-19	6.34 7.3*	0.75	1.3

\*  $N_{con}$  for soybean varieties intended for tofu

### 4.3.2 Sensitivity of the model

To demonstrate the model's sensitivity to the uncertainty of input variables, an arbitrary error factor was applied to each term of the model using faba bean as the model crop. Figure 13 depicts the propagation of errors affecting  $N_{fix}$  estimation. For instance, an error of +50% in estimating  $\alpha_{crop}$ ,  $N_{con}$ , or  $Y_{DM}$  leads to an overestimation of  $N_{fix}$  by approximately 113 kg ha<sup>-1</sup>. Conversely, a similar error magnitude (+50%) in estimating  $NHI$  results in an underestimation of approximately 76 kg ha<sup>-1</sup>. Notably, for faba bean, an underestimation of  $NHI$  by 50% leads to a significant overestimation of  $N_{fix}$  by 96%, while an overestimation of  $NHI$  by 50% only results in a -32% underestimation of  $N_{fix}$ . Although the pattern of the effect of varying input variables on  $N_{fix}$  estimation is consistent across all grain legumes, the degree of over or underestimation of  $N_{fix}$  slightly differs among crops. For instance, Anglade et al. (2011) reported a median value of 74% with an interquartile range of 63%-77% for  $NHI$  in faba bean, indicating that a 50% error is not realistic. However, for  $Y_{DM}$ , the variety test program results reveal wider variability for field pea or soybean from year to year and from site to site, hence we advocate conducting on-farm yield surveys ( $Y_{DM}$ )

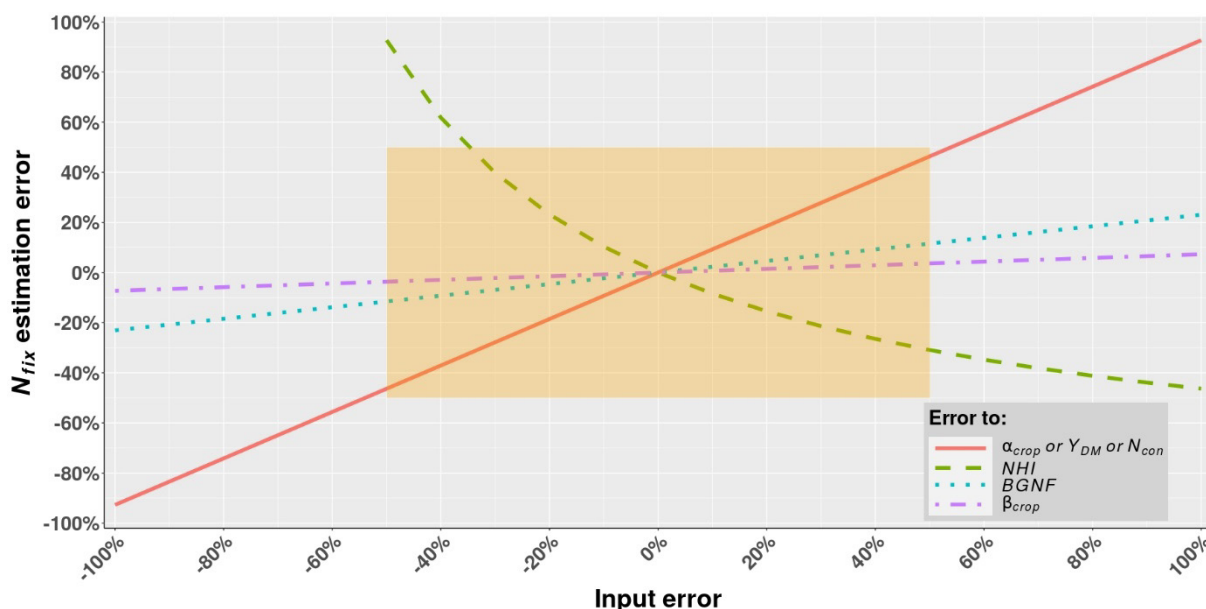


Figure 13. Sensitivity of the model for faba bean.  $Y_{DM} = 40 \text{ dt ha}^{-1}$ ;  $N_{con} = 4.46 \text{ kg N dt}^{-1}$ ;  $NHI = 0.74$ ;  $BGNF = 1.3$ ;  $N_{fix} = 237 \text{ kg N ha}^{-1}$ . The coloured rectangle represents a range of error of  $\pm 50\%$ .

#### 4.3.2.1 Utilization of the model with the results of the variety test program

The utilization of the model in this study appears to be straightforward, as the only parameter that needs to be directly collected on the farm is grain yield ( $Y_{DM}$ ), which can be obtained from farmers' cropping records (Feldkalender). In the following examples, we demonstrate the application of the model by using  $Y_{DM}$  and  $N_{con}$  values from Agroscope's variety test program, along with the values provided in Table 12 for the remaining parameters ( $\alpha_{crop}$ ,  $\beta_{crop}$ , and  $NHI$ ).

In the case of soybean, the Aveline variety exhibited the lowest mean  $N_{fix}$  value, recording 198 kg  $N_{fix}$  ha<sup>-1</sup> in Changins, while the highest value was observed for the Proteix variety in Reckenholz, with an  $N_{fix}$  value of almost 305 kg ha<sup>-1</sup> (Fig. 14). These values fall within the range reported by Salvagiotti et al. (2008) when considering the application of a  $BGNF$  of 1.3. Notably, at the Reckenholz site, the highest  $N_{fix}$  values were consistently obtained in 2016 and 2017 for all varieties, whereas Goumoens showed the lowest value in 2014, Giez in 2016, and Changins in 2017. Moreover, significant inter-annual variations were observed within the same site. For instance,  $N_{fix}$  for the best and worst year ranged from 25 to 150 kg ha<sup>-1</sup> for the Opaline variety at the Goumoens site, and for the Falbalà variety at the Reckenholz site, respectively.

Regarding field pea, the available database is smaller compared to soybean. Both the year of cultivation and the specific site had a considerable effect on the variation of  $N_{fix}$  (Fig. 15). Notably, the variety-specific differences in fixation between 2015 and 2016 were relatively smaller in Goumoens compared to Changins, where differences exceeding 100 kg  $N_{fix}$  ha<sup>-1</sup> were observed.

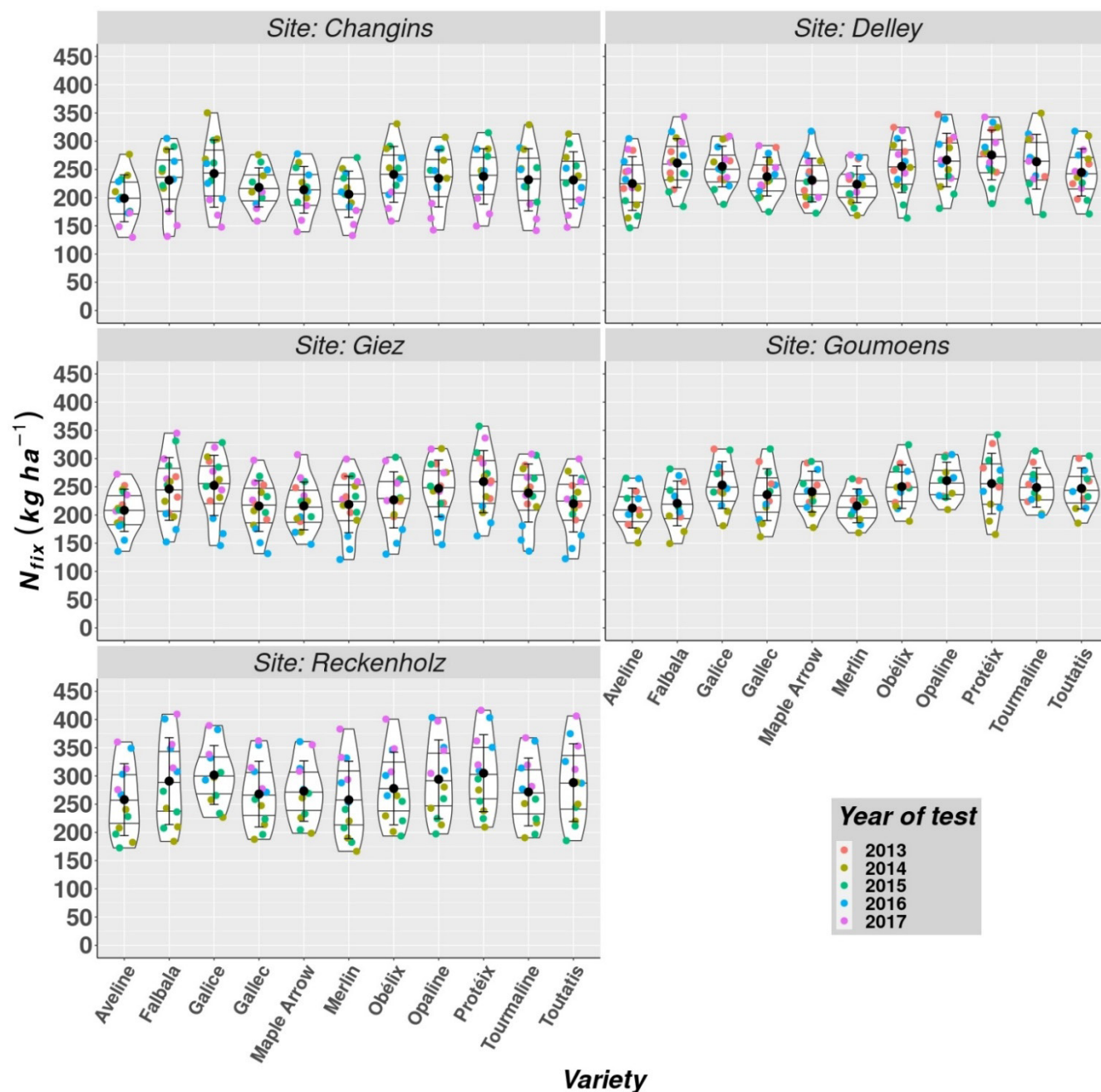


Figure 94.  $N_{fix}$  of soybean varieties according to the site and year of test. The violin shapes represent the kernel density: the larger the shape, the higher the density. The black line inside the violin represents the quartile (25%, 50% and 75%). Black dots and error bars represent the mean and standard deviation. Sources:  $Y_{DM}$ ,  $N_{con}$ : Agroscope variety test program;  $\alpha_{crop}$ ,  $\beta_{crop}$  and  $NHl$ : Salvagiotti et al. (2008). To highlight the variability due to the  $NHl$  all the three values proposed by them were used (mean and interquartile ranges, Tab. 11).

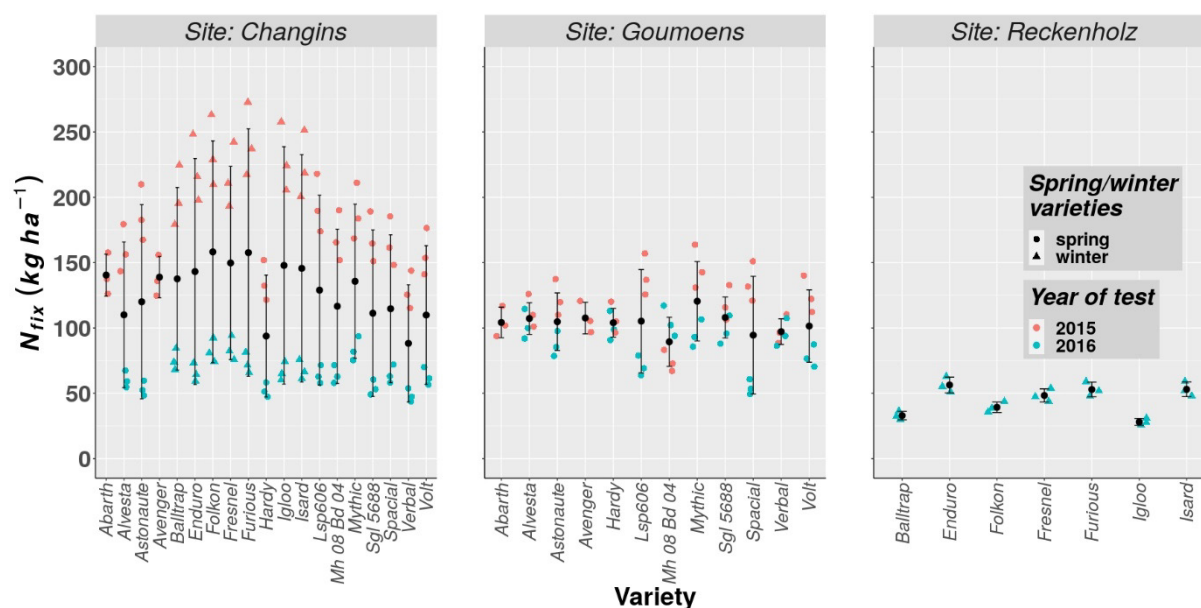


Figure 105.  $N_{fix}$  estimated for spring field pea varieties by site and year of test. Mean and standard deviation. Sources:  $Y_{DM}$ ,  $N_{con}$ : variety test program;  $\alpha_{crop}$ ,  $\beta_{crop}$  and  $NHI$ : Anglade *et al.* (2015). To highlight the variability due to  $NHI$ , all three values proposed by Anglade were used (median and interquartile range, Tab. 11).

### 4.3.3 Conclusions

Among the six input variables required by the model to estimate  $N_{fix}$  in grain legumes, only one variable ( $Y_{DM}$ ) needs to be determined in the field to ensure a robust estimation of  $N_{fix}$ . For the remaining variables, default values are proposed due to the lack of realistic alternatives. However, determining  $Y_{DM}$  can sometimes require additional effort from farmers or enforcement authorities, as it may not be a routine annotation in farmers' records, especially when harvested grains are used on the farm.

Nevertheless, the lack of data specific to Swiss conditions can impact the accuracy of the results. Specifically, updated values for  $NHI$  are currently unavailable for Swiss crops. To address this issue, the determination of  $NHI$  could be incorporated into the variety test program. This approach would allow for the assessment of differences between cultivars and lead to improved estimations. Similar considerations could also be made for the  $BGNF$ , as it remains a poorly investigated subject and the existing literature does not provide conclusive evidence for field conditions in Switzerland.

## 4.4 Cover crops

The use of cover crops has been a widespread practice since ancient times. Mixtures of legumes and non-leguminous species can be as effectively used as pure non-leguminous stands in preventing nitrate losses (Vogeler *et al.*, 2019). The use of legumes in cropping systems can reduce the dependence from synthetic N fertilizers, whose production (including products for industrial uses) emits globally 465 Tg of  $CO_2$  equivalents in global warming potential (IFA 2009). The attention of farmer and researchers on mixtures and pure leguminous stands is probably increasing because of these potential economical savings and the growing environmental awareness.

The available references for estimating  $N_{fix}$  from cover crops are scarce: the *AGFF Merkblatt 9* (Suter *et al.*, 2011) for grassland mixtures and Büchi *et al.* (2015) dealing with pure stands. The *AGFF Merkblatt 9* proposes a standard value for  $N_{tot}$  (above- and below-ground N uptake) according to mixture and seeding time. We synthesized the proposed values in table 13, simplifying the frame of reference.



Table 13. Dry matter yield, relative abundance of legumes, average  $N_{fix}$  and  $N_{tot}$ ; italic numbers are the min-max range according the seeding time (mid-August – mid-September) of the different standard mixtures (code numbers in the first column). Synthetized from Suter *et al.* (2011);  $N_{fix}$  has been calculated considering a soil mineral N content of 30 kg ha<sup>-1</sup>.

Standard mixture (SM)	$Y_{DM}^*$ (dt ha <sup>-1</sup> )	$Leg_{RA}$	$N_{fix}$ (kg ha <sup>-1</sup> )	$N_{tot}$ (kg ha <sup>-1</sup> )
<u>Non-overwintering</u> SM 101, SM 102, SM 106, SM 108	23 11 - 37	0.33	30 19 - 51	102 66 - 179
<u>Overwintering</u> SM 210, SM 151, SM 155, SM 200	15 2 - 34	0.25 - 0.33	18 3 - 44	73 13 - 178
<u>Green manure</u> <i>Trifolium subterraneum</i>		1.00	63 22 - 97	90 32 - 138

\*At the end of the autumn

Büchi *et al.* (2015) selected 19 legume species according to their actual or potential use as cover crops in Switzerland. They measured the  $N_{fix}$  in the above-ground parts with the natural abundance method after a growing period of three months in two different sites (Changins and Zollikofen) in 2011. The measured  $N_{fix}$  ranged from 2 kg N ha<sup>-1</sup> for chickpea (*Cicer arietinum*) in Changins to 172 kg N ha<sup>-1</sup> for faba bean in Changins (Tab. 14). The overall relation between AGN and  $N_{fix}$  is robust and allows to discriminate two main clusters:  $N_{fix}$  amount over 100 kg ha<sup>-1</sup> corresponding to an AGN of over 120 kg ha<sup>-1</sup>, recorded for common vetch (*Vicia sativa*), faba bean, pea and chickling pea (*Lathyrus sativus*), and  $N_{fix}$  amount lower than 100 kg ha<sup>-1</sup> for the other species, with a recorded AGN of less than 120 kg ha<sup>-1</sup> (Fig 16). Soybean (which in the practice is hardly used as cover crop) did not show high amounts of  $N_{fix}$  and particularly marked differences between the two considered sites have been recorded. Less pronounced but still relevant differences were also found for some of other species tested (Tab. 14).

Table 14. Characteristics of some of the cover crop tested by Büchi *et al.* (2015).

Crop	Latin name	Biomass (dt ha <sup>-1</sup> )		$N_{fix}$ (kg ha <sup>-1</sup> )		AGN (kg ha <sup>-1</sup> )	
		Changins	Zollikofen	Changins	Zollikofen	Changins	Zollikofen
Faba bean	<i>Vicia faba</i>	74.5	62.7	172	129	204	169
Chickling pea	<i>Lathyrus sativus</i>	39.9	29.5	149	101	161	129
Field pea	<i>Pisum sativum</i>	55.2	44.6	115	102	166	139
Common vetch	<i>Vicia sativa</i>	35.4	43.9	107	131	143	176
Soybean	<i>Glycine max</i>	48.3	34.1	55	4	143	60
White lupin	<i>Lupinus albus</i>	56.0	46.8	40	9	88	59
Chickpea	<i>Cicer arietinum</i>	30	20	3	2	22	9

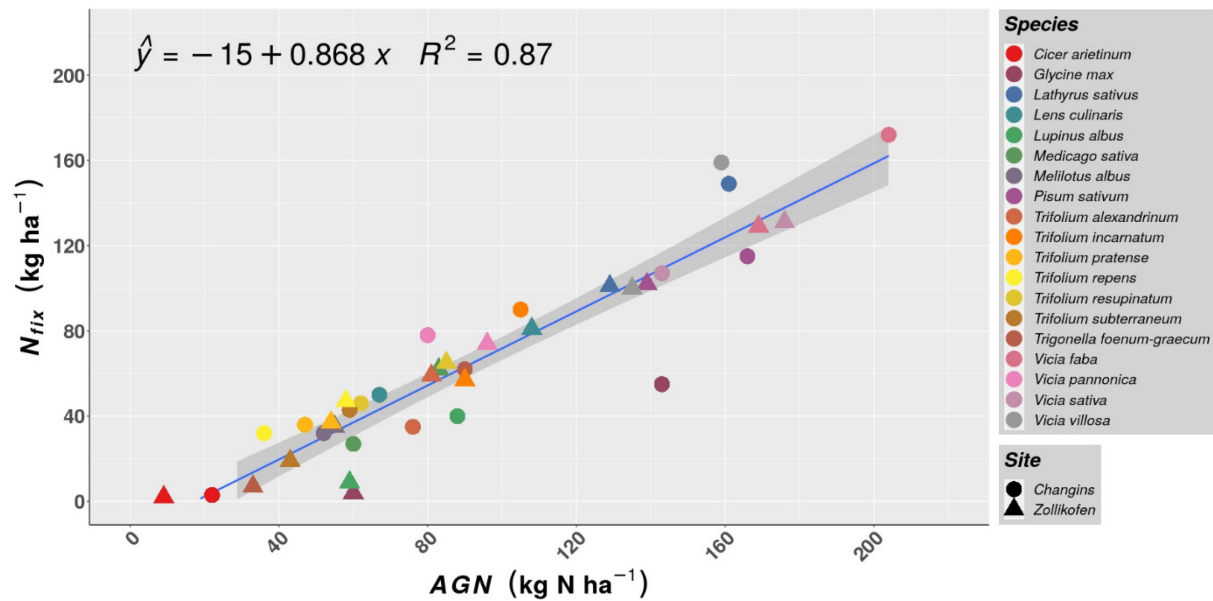


Figure 16. Relation between the AGN (kg ha<sup>-1</sup>) and the N<sub>fix</sub> of the 19 crops tested in pure stands by Büchi *et al.* (2015) in the sites of Changins and Zolllikofen in 2011. The shaded area represents a confidence interval of 95% of the regression.

The current Swiss farm balance system is not aimed to estimate nitrogen fixation and adopts a simplified approach considering three categories of yield instead (Agridea-FOAG 2018):

- 25 dt ha<sup>-1</sup> for grassland type cover crops per harvest, maximum two harvests
- 35 dt ha<sup>-1</sup> for green manure in arable farming
- 30 dt ha<sup>-1</sup> for leguminous green manure for horticulture

Due to ranges of variation of the  $Y_{DM}$  proposed in Suter *et al.* (2011, Tab. 13) and observed in Büchi *et al.* 2015, a simplified system like the one proposed in the current Swiss farm balance system is unsuitable for a correct estimation of the N<sub>fix</sub> by cover crops, either in mixtures or pure stands. However, due to scarce references, the values shown in table 13 for grassland type cover crops can be used with a large risk of bias. For pure legume stands and legume-nonlegume mixtures, we believe that the values proposed in Büchi *et al.* (2015) must be validated by other experiments under a broader range of environments before they can be used. Large variations of N<sub>fix</sub> can be expected due to the number of available cover crops, their mixing options (different species, legumes non-legumes mixtures), the duration and type of cover crops (late summer to spring of subsequent year; overwintering vs. winter-killed) and the yield development, which is strongly depending on yearly conditions (water availability, temperature). Hence, using default values will in any case result in a bias and a misjudgement of real N<sub>fix</sub>. So, DM yields or N uptake of cover crop legumes have to be estimated on individual fields. From the present knowledge, a practicable method with reasonable effort is not available for farmers. Estimations by remote sensing technologies might be available in a future perspective.

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