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Review

Challenges in Using Precision Agriculture to Optimize Symbiotic Nitrogen Fixation in Legumes: Progress, Limitations, and Future Improvements Needed in Diagnostic Testing

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Received: 12 March 2018; Accepted: 16 May 2018; Published: 21 May 2018

Abstract: Precision agriculture (PA) has been used for ≥25 years to optimize inputs, maximize profit, and minimize negative environmental impacts. Legumes play an important role in cropping systems, by associating with rhizobia microbes that convert plant-unavailable atmospheric nitrogen into usable nitrogen through symbiotic nitrogen fixation (SNF). However, there can be field-level spatial variability for SNF activity, as well as underlying soil factors that influence SNF (e.g., macro/micronutrients, pH, and rhizobia). There is a need for PA tools that can diagnose spatial variability in SNF activity, as well as the relevant environmental factors that influence SNF. Little information is available in the literature concerning the potential of PA to diagnose/optimize SNF. Here, we critically analyze SNF/soil diagnostic methods that hold promise as PA tools in the short–medium term. We also review the challenges facing additional diagnostics currently used for research, and describe the innovations needed to move them forward as PA tools. Our analysis suggests that the nitrogen difference method, isotope methods, and proximal and remote sensing techniques hold promise for diagnosing field-level variability in SNF. With respect to soil diagnostics, soil sensors and remote sensing techniques for nitrogen, phosphorus, pH, and salinity have short–medium term potential to optimize legume SNF under field conditions.

Keywords: legume; symbiotic nitrogen fixation; precision agriculture; sensors; satellite and UAS imagery; variable rate application; site specific management

1. Introduction

Legumes play a key role in adding nitrogen (N) through symbiotic nitrogen fixation (SNF) in annual legume crops [1], forage stands [2], and perennial agroforestry systems [3]. Rhizobia inside the root nodules fix the unavailable atmospheric N_2 into bioavailable N (NH_4^+), which is then assimilated into amino acids to enable protein production by the host plants [4]. As a result, legumes are rich sources of high quality protein for humans (e.g., lentil, beans, peanut) and livestock (e.g., alfalfa, clover, vetch) [5,6]. Legume crops can also enrich the soil N through organic N deposition in various cropping systems and crop rotations, when used as green manures or when a perennial legume is used [7–9]. However, legume crops growing in many agroecosystems suffer from suboptimal SNF, due to suboptimal biological factors (e.g., unavailability of compatible soil rhizobia or competition by indigenous rhizobia) and environmental factors (e.g., inadequate micronutrients, drought, high/low temperature, salinity, and pH). The major biological and environmental factors that challenge SNF in different legume crops have been comprehensively reviewed [10–16].

There is significant interest in using precision agriculture at the field scale to help growers optimize inputs and production while maximizing profits by developing proper decision support systems [17–19]. Typical practices used in precision agriculture include remote sensing, geographical information systems (GIS), spectral imaging, the global positioning system (GPS), data management, and sensors [18,20,21]. These techniques help to identify patterns of variability within a field to guide soil or plant sampling [22], which then directs optimal and rational spatial input use in conjunction with appropriate equipment (e.g., variable rate input applicators mounted on tractors). Precision agriculture technologies have been widely used for crop N management, especially with non-legumes (e.g., corn, wheat) [23–26]. However, little information exists in the literature concerning the potential of precision agriculture to optimize SNF.

Here, we critically analyze SNF diagnostic methods that hold promise as precision agriculture tools for legumes in the short–medium term. We similarly evaluate the potential of diagnostic methods used to measure soil traits that affect SNF. We also review the challenges facing additional SNF/soil diagnostics currently available, with respect to their potential to measure spatial variability within a field, in order to inspire researchers to innovate. Finally, we have proposed a long-term conceptual model that illustrates how various current and future precision agriculture diagnostics can be integrated to optimize legume SNF at the field scale.

2. Current SNF Diagnostic Methods That Have Potential as Precision Agriculture Tools

Different methodologies have been developed to quantify SNF in legumes under controlled and field environments [27–31]. Table 1 has summarized the current direct and indirect methods available to quantify SNF in legumes, categorized by their potential for adaption as precision agricultural tools in the short–medium term. Each method has its own strengths and limitations [30,31].

Table 1. Current SNF diagnostic methods and their potential for adoption as precision agriculture tools.

Method	Principle	Potential to Adapt to Precision Agriculture	
		Short–Medium Term	**Long Term**
Candidate precision agriculture diagnostics in the short-medium term			
Proximal and remote sensing [32]	Based on the near-infrared spectral region (NDVI or red-edge position (REP)) reflected by plant leaves/canopy. Canopy reflectance has positive correlation with leaf chlorophyll content.	Has tremendous potential to measure SNF at the field level, with the inclusion of control micro plots with different N fertilizer gradients applied within the field.	High probability.
Nitrogen difference method [28]	Based on the difference in shoot N accumulated by N_2-fixing plants vs. neighboring non-N_2-fixing plants (uninoculated or non-nodulating mutant). Assumptions: fixing and non-fixing plants absorb the same amount of N from soil, and surplus N in the fixing plant is from SNF.	Has potential for N-limiting soils if a non-nodulating reference legume genotype is available, and if spectral imaging can distinguish the reference genotype from N-fixing plants.	High probability.
^{15}N natural abundance method [28]	Takes into consideration the natural difference in δ^{15}N abundance between atmospheric N and soil N to measure SNF. SNF calculation based on the difference in δ^{15}N abundance between non-fixing reference plant (resembles soil δ^{15}N) and N-fixing plant.	Use of remote sensing technology to detect plant ^{15}N levels using leaf/canopy reflectance spectra may allow this technology to be used under field conditions.	High probability.
^{15}N isotope dilution method [28]	Plant available soil N is artificially enriched with ^{15}N-enriched fertilizers. N-fixing and non-fixing reference plants are grown in the soil with the same ^{15}N enrichment, and SNF calculated based on the difference in ^{15}N signature of the reference and fixing plants.	Use of remote sensing technology to detect plant ^{15}N levels using leaf/canopy reflectance spectra may allow this technology to be used under field conditions.	High probability.

Table 1. *Cont.*

Method	Principle	Potential to Adapt to Precision Agriculture	
		Short–Medium Term	**Long Term**
Diagnostics requiring major advances to adapt them for precision agriculture			
Nitrogen balance method [28]	Calculates the difference between all N inputs (e.g., N-fixation, manure, mineralization, inorganic N) and N outputs (e.g., leaching, volatilization, crop removal, erosion) over a period of time.	Low probability: challenging due to the requirement to measure N in multiple components over a long period of time.	Advances are being made in data collection (e.g., satellite images) and big data management using different software programs.
Ureide method [33]	Based on the % ureide N in the xylem sap or stem segments.	Low probability: challenging due to requirement to calibrate different crops, under different growth stages as well as under different stress conditions.	A high throughput low-cost method is needed to analyze ureide compounds (e.g., amperometric biosensor specific for ureides).
C_2H_2 reduction assay (ARA) [34]	Based on the promiscuous activity of nitrogenase enzyme, which can reduce acetylene to ethylene which is then measured by gas chromatography; provides an estimate of N_2 fixation activity at a point in time.	Low probability: challenging to measure gas compound under field conditions.	Low probability.
Nodule number, dry weight, color [29,35–37]	- Manual counting and observation of nodules - Manual separation and dry weight measurement - Software-based analysis of scanned root systems for nodule number	Low probability.	Challenging: need for futuristic underground X-ray and magnetic resonance imaging like technologies to examine root nodule density.
GlnLux method [31]	*GlnLux* assay is based on measuring glutamine output of leaf punch extracts using the *GlnLux* biosensor, which consists of *Escherichia coli* cells auxotrophic for glutamine. The assay can be used to measure SNF output of legumes grown under controlled conditions with minimal external N supply.	Low probability.	Holds potential if converted into a field-based technology such as translation into an amperometric biosensor specific for Gln.
Chlorophyll meter [38]	The SPAD (soil plant analysis development) index gives an indirect measure of leaf chlorophyll. Leaf SPAD index can be used to measure relative SNF under axenic conditions with limited N supply.	Has potential on N-limiting soils if a non-nodulating reference legume genotype is available, and if SPAD can distinguish the reference genotype from N-fixing plants.	Has potential on N limiting soils with a non-nodulating reference genotype.

2.1. Shoot Nitrogen Status

Among the diagnostic targets reviewed in this paper, shoot N status has received the greatest attention from commercial precision agriculture. Sensors have been developed using spectroradiometers, reflectometers, imagery from satellite sensors, and digital cameras to detect the N status of plants at the leaf and canopy level [39,40]. These methods have potential for measuring SNF under field conditions (Table 1). The most common systems that have been used to test plant N status are the Soil Plant Analysis Development (SPAD) meter (based on foliar chlorophyll content) and Normalized Difference Vegetative Index (NDVI) (based on the near-infrared spectral region reflected by plant leaves) [32,41,42] (Table 1). SPAD measures the plant N status at the leaf level, whereas NDVI is a canopy-level diagnostic. Some commercially available NDVI devices are the hand-held FieldSpec spectro-radiometer sensor (Analytical Spectral Devices, Inc., Boulder, CO, USA), CropScan (Next Instruments, Condell Park, New South Wales, Australia), LI-COR portable spectroradiometer (LI-COR Inc., Lincoln, NE, USA), tractor-mounted Yara N-Sensor (Yara International ASA, Oslo, Norway), hand-held GreenSeeker (NTech Industries Inc., Ukiah, CA, USA), tractor-mounted GreenSeeker TM (Trimble, Westminster, CO, USA) and hand-held Crop Circle (Holland Scientific, Lincoln, NE, USA) [39]. NDVI is currently being replaced by the Normalized Difference Red Edge Index (NDRE) for field crop N management, as NDVI becomes saturated and is overly affected by leaf area index (LAI), whereas NDRE is not easily saturated and is more sensitive to chlorophyll content [43,44]. NDRE is readily available for field-level variable rate use, including with Crop Circle [44]. Hyperspectral reflectance images have also been used to measure plant N status [45–48]. Satellite images that are taken by space mounted devices (QuickBird, Ikonos, Hyperion, Proba CHRIS) are also used to sense plant N-status at the field level [20]. Additional precision hyperspectral sensors such as EnMap, HyspIRI, RapidEye, WorldView-2, and SumbandilaSAT are also emerging as tools to measure canopy N status. However, small unmanned aerial systems (UAS) are becoming more popular for taking images at a low altitude, due to low operational costs, high operational flexibility, and high spatial resolution of the images [20,49]. The above sensors can be categorized as active (e.g., GreenSeeker, Crop Circle) or passive (e.g., imagery from satellite/drones or spectral radiometers), depending on whether the sensor system uses sunlight (passive sensor) or is equipped with light-emitting devices (active sensors) as the light source [50].

One challenge with the above technologies is that canopy N status, measured using crop canopy reflectance, leaf transmittance, chlorophyll, and fluorescence, can suffer from interference from plant physiological status, sunlight variation, soil conditions, and chlorophyll saturation [39]. In particular, passive sensors have limitations due to intermittent cloud cover/cloud-free days, bidirectional reflection issues associated with solar angle, and a short time window for operation [39,51,52]. NDRE with an active light source can overcome some of these problems [44]. Furthermore, use of high N reference areas with the same variety can overcome some physiology-associated problems.

A major additional problem is that legumes have two main input sources of N: N taken up from the soil and N derived from the atmosphere through SNF. Both sources of N result in the same biochemical end-traits within the plant (e.g., chlorophyll), and as a result the non-SNF-derived N interferes with the above-mentioned precision agricultural tools (e.g., SPAD, NDVI) for accurate SNF diagnosis under field conditions. To overcome this problem, inclusion of random micro trials (e.g., micro plots or strip plots) within a given field that control for this challenge (e.g., non-nodulating genotype, uninoculated plants, and the gradient rate of applied inorganic N) can be used to extrapolate the contribution of SNF under field conditions, in order to calibrate the above-mentioned precision agricultural tools.

A final major challenge of the above techniques is that they rely on leaf chlorophyll; however, chlorophyll is relatively stable and does not change dynamically throughout the growing season at the same pace as SNF and soil N availability (e.g., due to mineralization, inorganic fertilizer mobility). To mitigate this challenge, rapid analytical methods are needed that measure N metabolites in plant sap, which changes on an hourly/daily basis [53,54] in order to make rapid decisions about SNF activity. Ultimately, it may be useful to conduct real-time soil mapping using precision agricultural techniques to diagnose soil N availability at the field scale (see below).

2.2. Nitrogen Difference Method

This method currently uses analytical chemistry to measure the difference in N accumulation between SNF plants and non-fixing reference plants. In order to translate the approach to precision agriculture, spectral imaging and other emerging technologies would need to be calibrated, which may be challenging (Table 1). Furthermore, the method may be problematic in soils with moderate to high levels of mineral N, which suppresses SNF activity, or when the root morphology is different between fixing and non-fixing plants, as this will result in different rates of background soil N uptake [30]. However, the method holds potential for resource-limited soils, if two conditions are met: first, if a non-nodulating reference legume genotype is available; and second, if spectral imaging can distinguish the reference genotype from SNF-active plants. Micro-plots of non-nodulating reference plants would need to be interspersed within the field. With these improvements, the method has a high probability of being converted into a precision agriculture tool to measure SNF in the short-medium term.

2.3. Nitrogen Isotope-Based Methods

SNF activity alters the $^{15}N/^{14}N$ ratio in plant tissues; as a result, this ratio differs between SNF-active plants and non-fixing control plant tissues (Table 1) [27]. Currently, N isotope-based methodologies (natural abundance and isotope dilution methods) are commonly used as precision methods to quantify SNF under field conditions (Table 1), but they require samples to be sent to a laboratory for mass spectrometry. The methods involve labor-intensive plant sample preparation before the analysis (e.g., sample grinding, encapsulation of ground samples), and the isotope determination is expensive (e.g., $10–15 USD per sample), and thus impractical for elucidating field level variation in SNF. To adapt this method for precision agriculture, there may be potential to circumvent the above costs, labor, and laboratory requirements through the use of remote sensing, which has been employed to detect plant ^{15}N levels using leaf/canopy reflectance spectra [45,55–57]. With these new technologies, the ^{15}N based methods hold promise as precision agriculture tools to measure SNF in the short–medium term.

3. Current SNF Diagnostic Methods That Require Major Technical Advances to Facilitate Their Application to Precision Agriculture

In addition to the above promising methods, Table 1 also summarizes additional methods that are commonly used for SNF diagnostics by researchers, but which have significant technical barriers that will prevent their adoption for precision agriculture in the near future. We discuss the limitations below in order to challenge the research community to come up with new innovations to overcome them.

3.1. Nitrogen Balance Method

The nitrogen balance method is complex, as it calculates N input versus output at the system level, including soil mineralization, soil organic matter, and harvest yield (Table 1). To translate this approach into an SNF precision agriculture tool, different components related to field N inputs and outputs would need to be accurately measured over an extended period of time [30], which may be challenging, though advances are being made in using satellite imaging, for example to measure soil organic matter [58]. Inaccurate measurements of any component can result in overestimation or

underestimation of SNF. In the short–medium term, the method has low probability for adaptation into a precision agriculture tool for measuring SNF.

3.2. Ureide Assays

In ureide-exporting legumes (e.g., soybean, cowpea), N fixed by rhizobia inside root nodules is assimilated into the amino acid glutamine (Gln), and further metabolized into ureide compounds (allantoin and allantoic acid) for long-distance transport within the plant via xylem [59]. Currently, in this assay, xylem sap compounds from stems and petioles are analyzed in a laboratory [33] (Table 1). Although a colorimetric method has been developed as an alternative to traditional methods for analyzing ureide concentrations in ureide-exporting legumes (e.g., soybeans), multiple, laborious steps have limited its use as a precision analytical method [60]. To adapt this approach for precision agriculture, a high throughput method is needed to analyze ureide compounds in the field (e.g., an amperometric biosensor specific for ureides) or a simpler laboratory assay to permit high-density, spatial sub-sampling of plant tissues from a field. However, precautions have to be taken when evaluating SNF data using this method, as xylem ureide concentrations can vary under plant stress conditions, as well as during different growth stages of the plant [30,61]. As a result of these challenges, the ureide assay has low probability of being adapted into a high-throughput SNF precision method in the short–medium term.

3.3. Acetylene Reduction Assay

The acetylene reduction assay (ARA) involves a laboratory-based gas chromatography instrument to measure the promiscuous reduction of acetylene to gaseous ethylene by rhizobia nitrogenase [34] (Table 1). Although ARA was developed 50 years ago [34], it is still being used to measure N fixation, but under short time frames [62–65]. ARA is commonly used under controlled environments [66], as well as for root nodule samples collected from field grown plants [67]. The ARA only has potential for use as a high-throughput SNF precision method under controlled conditions in the short–long term when implemented using continuous flow-through incubations and spectral monitoring of acetylene [64]. This technology is unlikely to be adopted to measure field-level variability in SNF until new innovations are achieved.

3.4. Nodulation Traits

One primary plant trait that has been used to test SNF ability in legumes is nodulation ability [13]. Indirect methods, such as measuring nodule number, nodule dry weight, nodule color (presence of leghemoglobin), and nodule distribution within root systems have been widely studied to evaluate legume-rhizobia symbiosis [11] (Table 1), but the main drawback associated with these methods is their labor-intensive nature. Development of simple, high-throughput nodulation diagnostics are needed (Table 1). X-ray computed tomography (CT) and magnetic resonance imaging (MRI) techniques have the potential for non-destructive 3D mapping of root systems in soil [68–70], to explore nodulation patterns. However, it is important to note that nodule parameters show only a modest correlation with SNF activity [13]. In general, these technologies are likely unfeasible as precision agriculture tools in the short–medium term.

3.5. GlnLux Assay

Whole cell biosensors are engineered to detect a specific metabolite and then emit a measurable output (e.g., photons) [71]. A microbial biosensor, *GlnLux*, was recently reported which detects glutamine (gln) from leaf punch extracts under laboratory conditions [31]. *GlnLux* is a rapid, inexpensive, high-throughput, and relatively non-destructive method for measuring SNF output in amide-exporting legumes (e.g., lentil, alfalfa) and ureide exporting legumes (e.g., soybean, cowpea) (Table 1). However, this method has limitations for use under field conditions in the short–medium term, as the *GlnLux* method cannot distinguish N derived from SNF versus soil-uptake N. This method has potential for precision agriculture if translated into a field-based technology, by conversion into an amperometric biosensor specific for gln [72] that can be linked to variable rate fertilization. There is also potential to engineer additional amperometric biosensors that can detect other fixed-N metabolites [73], as well as inorganic N [74]. Chemical-based tests are currently available (e.g., Merkoquant test strips, Reflectoquant strips) to measure plant nitrate concentration from plant sap at the field level [39].

4. Opportunities to Use Precision Agriculture to Diagnose Soil Traits That Affect SNF

Under the concept of precision agriculture, farmers' fields are no longer considered as homogenous units, but rather as heterogeneous entities. As SNF is affected by multiple biotic and abiotic factors related to the soil, there are additional opportunities to diagnose and optimize SNF at the field scale, using precision agriculture diagnostics that target soil traits. In particular, growers can experience spatial variability in SNF efficiency within a field, due to variations in nodule occupancy by different rhizobia strains, micronutrients, available soil N/P, and soil pH [13]. New precision agricultural tools are available to test the spatial variability in soil for different chemical traits (e.g., N, pH, EC, organic matter) and physical parameters (e.g., bulk density, moisture) using sensor-based and map-based approaches [21,75]. These diagnostics may facilitate mitigating management practices (e.g., inorganic N fertilizer application, liming, tile drainage). Moving into the future, development of on-the-go in situ measurements for available soil macronutrients (e.g., N and P), micronutrients (e.g., Mo, B), and soil pH are needed to support site-specific management of soil chemical properties, and to permit a variable-rate input application for field-level SNF optimization (Table 2).

Table 2. The precision agriculture adaptability of current diagnostic methods for soil traits that impact symbiotic nitrogen fixation.

Soil Trait	Current Diagnostic	Potential to Adapt to Precision Agriculture	
		Short–Medium Term	**Long-Term**
Candidate precision agriculture diagnostics in the short-medium term			
Available soil nitrogen [76,77]	• *Soil nitrate test* - Nitrate electrode method - Cadmium reduction method • *Soil ammonium test* - Ammonia electrode method - Colorimetric methods	High probability: • *Spectrophotometric/spectroscopic techniques* - Near-infrared reflectance spectroscopy (NIRS) - Mid-infrared Fourier transform attenuated total reflectance (ATR) spectroscopy - Morphology-dependent stimulated Raman scattering (MDSRS) • *Electrochemical techniques* - Nitrate ion-selective field-effect transistor (ISFET) - Nitrate combination (CCR-ISE) • *Biological techniques* - Nitrate biosensors (BS)	High probability.
Available soil phosphorus [78]	- Olsen - Bray and Kurtz P1 - Mehlich 1 - Mehlich 3	High probability: • *Spectrophotometric/spectroscopic techniques:* - Raman scattering (RS) - Reflectance spectroscopy (NIRS) • *Electrochemical techniques* - Phosphate ISE - Phosphate coated wire field-effect transistor (CW/FET) • *Biological techniques* - Phosphate biosensors (BS)	High probability.

Table 2. *Cont.*

Soil Trait	Current Diagnostic	Potential to Adapt to Precision Agriculture	
		Short–Medium Term	**Long-Term**
Soil pH [79,80]	- Electrometric method with $CaCl_2$ or water extracts - Solid state pH electrode (ISFET) (in situ method)	High probability: - Visible and near infrared (VIS/NIR) diffuse reflectance spectroscopy	High probability.
Salinity [81,82]	- Electrical conductance of soil solution extracts - In situ electrical resistivity measurement (ER) - Non-invasive measurement of electrical conductance using electromagnetic induction (EM) - In situ measurement of electrical conductance with time domain reflectometry (TDR)	High probability: - Multispectral satellite sensors - Hyperspectral sensors - Vegetation indices [e.g., NDVI, soil adjusted vegetative index (SAVI), ratio vegetative index (RVI), brightness index (BI), green vegetation index (GVI) and wetness index (WI)]	High probability.
Diagnostics requiring major advances to adapt them for precision agriculture			
Competitive soil rhizobia [13]	- Nodule occupancy test based on PCR based taxonomic methods	Low probability.	- Need for high-throughput nodule occupancy taxonomic diagnostic tests.
Available soil molybdenum [83]	- Soil extracts tested with potassium iodide plus hydrogen peroxide (KI + H_2O_2 reaction) - Soil extracted with acid ammonium oxalate buffered at pH 3 - Soil extracted with ammonium bicarbonate DTPA (AB-DTPA) - Molybdo-thiocyanate method - U.S. EPA method 350	Low probability.	- In situ sensor based measurements for available soil molybdenum needed. - Development of spectral-based quantitative assays for Mo needed.
Available soil boron [84]	- Hot-water-soluble boron test - HCl extracted boron - Ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) extraction [Soil extracts are analyzed for B with colorimetric methods (turmeric test, curcumin method, carmine method, azomethine-H method) and spectrometric methods]	Low probability.	- In situ sensor based measurements for available soil boron needed. - Development of spectral—based quantitative assays for boron needed.

4.1. Current Soil Tests That Hold Promise as Precision Agriculture Tools to Optimize SNF

4.1.1. Available Soil Nitrogen

High N availability in soil significantly reduces the nodule number [13] and suppresses SNF activity in legume crops, based on feedback inhibition [85]. Application of inorganic N fertilizer to areas with high soil N availability not only reduces SNF rates, but can also lead to nitrate leaching, resulting in economic losses and environmental pollution. Therefore, it is important to identify the variability in soil N availability, especially residual N [22], within an agricultural field to optimize SNF. Plant tissue N diagnostics were described above (Table 1). Available soil N is mainly measured by quantifying nitrate and ammonium. However, the different soil N diagnostic methods currently available are tedious, as they involve soil sample collection, extraction, and multi-step analysis (Table 2) [77]. Also, the concentration of available N in the soil can change rapidly throughout the growing season based on environmental conditions (e.g., soil moisture) [86], therefore monitoring this temporal variability is critical. Fortunately, in situ, on-the-go N determination techniques have been developed based on spectrophotometric/spectroscopic, electrochemical, and biosensor techniques (Table 2) [87,88]. The underlying mechanisms of these methods (NIRS, ATR, MDSRS, ISFET, CCR-ISE, and BS) (Table 2) are described in detail by Sinfield et al. 2010 [88]. Some of these techniques are already commercially available (e.g., NIRS), while others are in progress to be developed as precision diagnostic techniques for field application [88]. However, some of the suggested diagnostic tests are challenging for use under field conditions (e.g., ATR, MDSRS, CCR-ISE), requiring new technical advances. As biosensor methods (for N) are very sensitive, respond quickly, and are highly precise and accurate, they have potential for precision agriculture, but primarily for testing soil and plant tissues at a high sample density to provide field-level spatial resolution [88]. Soil organic matter content is an indicator of potential soil organic N mineralization, and the NIRS technology can be used for on-the-go mapping. All of these suggested diagnostics can help to generate field soil maps that report soil N variability, in order to enable variable rate application or site-specific management of N fertilizer for non-legume crops, to maximize the benefits of SNF.

4.1.2. Available Soil Phosphorus

Phosphorus (P) is an important macronutrient for nodulation and SNF in legumes. P deficiencies in soil reduce nodule growth, nodule number, and nodule activity [89,90]. Plants mainly take up P in the form of orthophosphate. Current available methods for soil phosphate are restricted to laboratory-based assays (Table 2) [78]. In order to monitor P dynamics in soil, in situ, real-time phosphate determination techniques are needed. Similar to available N, spectrophotometric/spectroscopic, electrochemical, and biosensor-based techniques have been developed (Table 2) [87,88], which are likely feasible to adapt for precision agriculture in the short–medium term. The underlying mechanisms of these methods (RS, NIRS, ISE, CW/FET, BS) (Table 2) are described in detail by Sinfield et al. 2010 [88]. Compared to soil N tests, less research has been conducted to develop on-the-go phosphate sensors. According to Sinfield et al. 2010, electrochemical methods hold more future potential for on on-the-go phosphate measurements [88]. Some of the phosphate diagnostic sensors are challenging to use under field conditions, due to interference by other soil elements and their short lifetime (e.g., phosphate ISE, CW/FET), and thus advances in research are needed to overcome these challenges. Although phosphate biosensor methods are very sensitive and accurate, advances are needed to overcome their short lifetime, instability, and lack in robustness to make them into commercial on-the-go diagnostic tools [88]. Variable-rate P fertilization has been shown to improve P management in different crops, including corn, wheat, and soybean [91–93]. Dynamic field mapping of phosphates can be performed using the data gathered from the above sensors, to enable variable rate application of P to optimize SNF. Alternatively, P deficiencies can be diagnosed using spectral imaging of plants [94] and colorimetric analyzers based on mobile phone cameras [95].

4.1.3. Soil pH

Most legume crops require neutral to slightly acidic pH to maintain SNF at optimal rates [10]. Acidic or alkaline conditions can have negative impacts on rhizobia survival in soil [13], nodulation, and nodule activity [10]. Therefore, it is important to diagnose variability in soil pH within an agricultural field, to permit variable-rate pH adjustment (e.g., liming). Electrometric methods with $CaCl_2$ or water extracts are traditionally used for pH measurement, which require soil sample collection, extraction, and pH meter reading (Table 2). In addition to electrometric methods, solid-state pH electrodes have been successfully used for in-situ measurement of soil pH [79,96]. In this method, soil cores are analyzed within the field, using pH electrodes that are mounted into a moving vehicle [96]. However, visible and near infrared (VIS/NIR) diffuse reflectance spectroscopy have potential for use as on-the-go tools to measure soil pH in a manner that is rapid, accurate, and low-cost [97–99]. VIS or NIR spectrophotometers can be mounted on a GPS-enabled tractor to measure soil spectra within a given field (on-line measurements) [99]. Soil penetration units (subsoiler) enable optical probes to acquire soil spectra from the bottom of an open trench [98]. The suggested technologies are feasible to adapt into a precision agriculture diagnostic in the short–medium term.

Liming is considered to be the most effective method of overcoming soil acidity [12], where variable-rate liming has been adopted to reduce the pH variability within a given field for different crops, including corn, wheat, and soybean [92,100,101]. Therefore, new precision agriculture technologies that measure soil pH can be used to identify and manage the soil pH variability using variable-rate lime recommendation maps, with the goal of optimizing SNF.

4.1.4. Salinity

Salinity is a major challenge for agriculture in some regions of the world (e.g., semi-arid region) [102], where it negatively affects rhizobia survival in soil and the initiation/maintenance of legume-rhizobia symbiosis [10,13]. Traditionally, soil salinity has being measured using electrical conductance of soil solution extracts, electrical resistivity (ER), electromagnetic induction (EM) and time domain reflectometry (TDR) (Table 2) [81,82]. Soil salinity has been mapped based on precision agricultural methods, including multispectral satellite sensors, hyperspectral sensors, and vegetation indices, e.g., NDVI, the soil adjusted vegetative index (SAVI), the ratio vegetative index (RVI), the brightness index (BI), the green vegetation index (GVI), and the wetness index (WI) [103]. Spatial as well as temporal mapping of salinity help in site-specific management to mitigate this major environmental problem. With respect to legumes, in the future, site-specific management of inputs (e.g., irrigation, fertilizer type, salinity-resistant varieties) can be used to manage the salinity within an agricultural field to optimize SNF. These technologies have a high probability of being adapted for use as precision agriculture tools in the short–medium term.

4.2. Current Soil Diagnostics That Require Major Technical Advances to Facilitate Their Application as Precision Agriculture Tools for SNF Optimization

4.2.1. Soil Rhizobia Detection

Rhizobia inoculants face challenges. First, introduced inoculants can be out-competed by native rhizobia strains that reside in the soil, which then occupy nodules, and hence field-level variability for these competitive strains alters the efficacy of inoculants [13]. Therefore, it is important to identify the presence of an indigenous rhizobia population, as well as nodule occupancy by the indigenous and introduced rhizobia (inoculants) within a given field, in order to improve the efficacy of the rhizobia inoculants for SNF [104]. Second, the key to successful nodulation by introduced rhizobia (inoculant) is the number of viable rhizobia available for the infection of legume roots [13]. Seed-applied rhizobia can lose their viability due to desiccation, temperature, seed coat toxicity, and pesticides [13,105]. To diagnose whether nodules were successfully colonized by an inoculant, and to diagnose nodules occupied by native rhizobia, whole-genome PCR-based taxonomic diagnostics are currently available

(e.g., BOX-PCR) [106]—but these are expensive and labor-intensive. Thus, high-throughput nodule occupancy diagnostic tests are needed for growers (Table 2), but these are difficult to imagine at a field scale in the short–medium term. Additionally, there is variability between nodules within a single root system for SNF activity [107], which is currently difficult to diagnose without using genetically engineered rhizobia. Therefore, a high-throughput SNF-diagnostic technology with nodule-scale resolution is needed—again, technically challenging at the field-level scale. Therefore, major advances are needed for rhizobia detection to become a diagnostic for precision agriculture. The most feasible approach to incorporate rhizobia diagnostics may be to conduct replicated on-farm strip trials with field scale equipment, to compare inoculated and non-inoculated strips, and then use NDRE and yield sensors to assess within-field variation in response to inoculation. This can be accompanied by diagnosis of causes of low SNF for the determination of future corrective actions.

4.2.2. Available Soil Micronutrients

It was reported that soil B deficiencies have been detected in nearly 80 countries, whereas Mo deficiencies predominate in Australia, China, Africa, and some part of India and the USA [108]. This problem has become exacerbated with the introduction of high-yielding new crop species and varieties, which have higher requirements for micronutrients. Micronutrients (specially Mo and B) are important for the legume–rhizobia symbiosis (e.g., Mo is a co-factor for nitrogenase), and hence soil micronutrient deficiencies reduce the potential N credit from SNF [109]. This is a common problem in developing countries, where laboratory facilities are not available to test soil micronutrient availability. Even in developed countries, a single soil test for Mo can be expensive. Currently, soil micronutrient mapping (e.g., B and Mo) is performed through chemical analysis of the soil samples [110], which have a very low probability of becoming the basis of precision agriculture based diagnostics. Instead, major technical advances are needed to develop low-cost in situ-based methods to diagnose field level variability in relevant micronutrients (e.g., spectroscopic) (Table 2), allowing variable-rate micronutrient application to optimize SNF. However, soil Mo and B are probably best assessed with grid soil sampling, unless their availability to plants and SNF are expected to vary greatly and unpredictably over time. Progress has been made on high throughput microscale digestive systems [111], which can be used for rapid sample preparation prior to chemical analysis. Furthermore, as soil Mo availability is limited in acidic soils [108], variable-rate liming can be adopted to reduce the pH variability within a given field (see Section 4.1.3) and simultaneously improve soil Mo availability, in order to optimize legume SNF. If a legume responds to B or Mo (both yield and SNF), it is probably best to regularly apply the micronutrient(s) to targeted zones within a field.

5. Perspectives on the Future of Precision Agriculture to Improve SNF in Field Legumes

A conceptual model is presented (Figure 1) of how different precision agricultural tools can be integrated to capture relevant field level heterogeneity, in order to improve legume SNF using on-the-go variable management. It is important to determine whether SNF is low within a given field, and then determine the cause of low SNF (e.g., biotic or abiotic factors). It is imagined that diverse soil chemical and physical parameters will be collected using soil core analytics (e.g., using zone or dense-grid soil sampling), as well as future underground monitoring sensors that are installed in the field at high density, but especially satellite/UAS images. Cloud-based servers will be used for data storage. Soil sub-sampling of zones or dense grids within a field has been widely used to accurately measure different soil chemical and physical traits, and to enable site-specific nutrient management [112,113]. Similarly, various canopy parameters (e.g., plant N status) will be collected using future tractor mounted/hand-held sensors (e.g., GreenSeeker NDVI) or satellite/UAS images. In the distant future, it is hoped that major breakthroughs will permit nodule occupancy/taxonomy to be evaluated, to monitor efficacy of rhizobia inoculants, and to mitigate competitive soil rhizobia. All the input data will be integrated to generate a prescription map for feeding into a tractor-mounted

computer system to enable variable rate application or site-specific management of inputs required to optimize legume SNF.

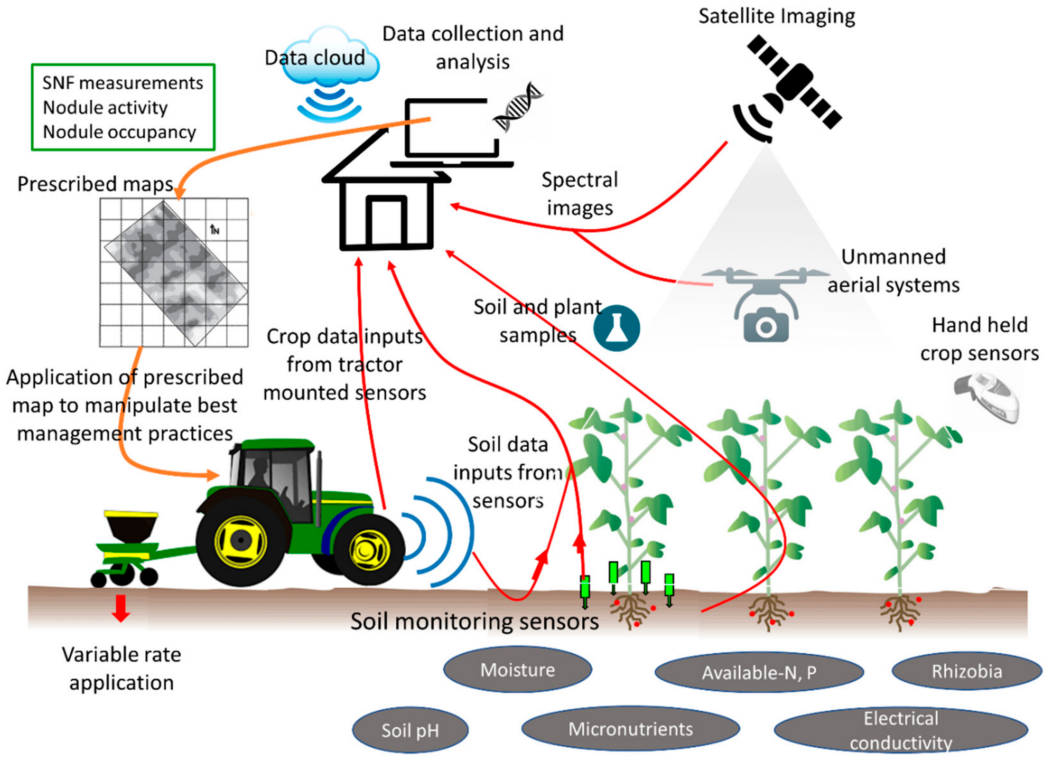

Figure 1. A conceptual model showing how different precision agricultural tools can be integrated together to improve symbiotic nitrogen fixation (SNF) in legumes at the field level, using on-the-go variable management. It is envisioned that soil chemical and physical properties will be collected using improved soil monitoring sensors, sensors mounted on tractors and soil core analytics. Canopy parameters (e.g., plant N status) will be collected using novel tractor mounted/hand-held sensors (e.g., similar to GreenSeeker NDVI) or satellite/UAS images. It is hoped that breakthrough technical advances will permit nodule occupancy to be measured using a high throughput rhizobia taxonomy detector. Nodule activity and nitrogen derived from the atmosphere will be measured using field collected samples. Sensor-based measurements of the legume crop will be combined with the spatial maps, which will be developed using the satellite or airborne systems in order to program variable input application rates based on their variability within a field.

A flow chart is presented (Figure 2) that describes how prescription maps can be generated using different precision agricultural tools, considering the variability within a given field (management zone approach). As the first step, spectral imaging (e.g., NDVI images) of the field can be captured using drone/satellite images coupled with GPS mapping. Using different software algorithms (e.g., Climate FieldRevealTM), management zones within a field can be generated using vegetative reflectance (e.g., NDVI), yield data, and soil data, where zones within a field can be categorized as low/medium/high performance zones, or to even a finer scale based on the field-level variability. Furthermore, soil fertility, soil electrical conductivity (collected from sensors), soil pH, topography, and historical crop management information can be used to improve the existing map. Data on soil nutrient parameters (e.g., mineral N, available P) across the field can be precisely measured by analyzing zone-specific soil samples, where fertility response curves can be used to determine how much fertilizer should be added to each zone. As the final step, prescription maps can be generated using different software programs (e.g., SMS) for input into GPS-enabled tractor mounted computers to enable variable rate application of inputs. Specifically with legumes, prescription maps can be

generated to mitigate the variability within a field through the application of macronutrients (e.g., N or P), micronutrients (e.g., B, Mo), and other soil amendments (e.g., biochar) to optimize SNF rates. In addition, in the future, it is hoped that crop genotypes can be matched with the available soil rhizobia to optimize SNF rates.

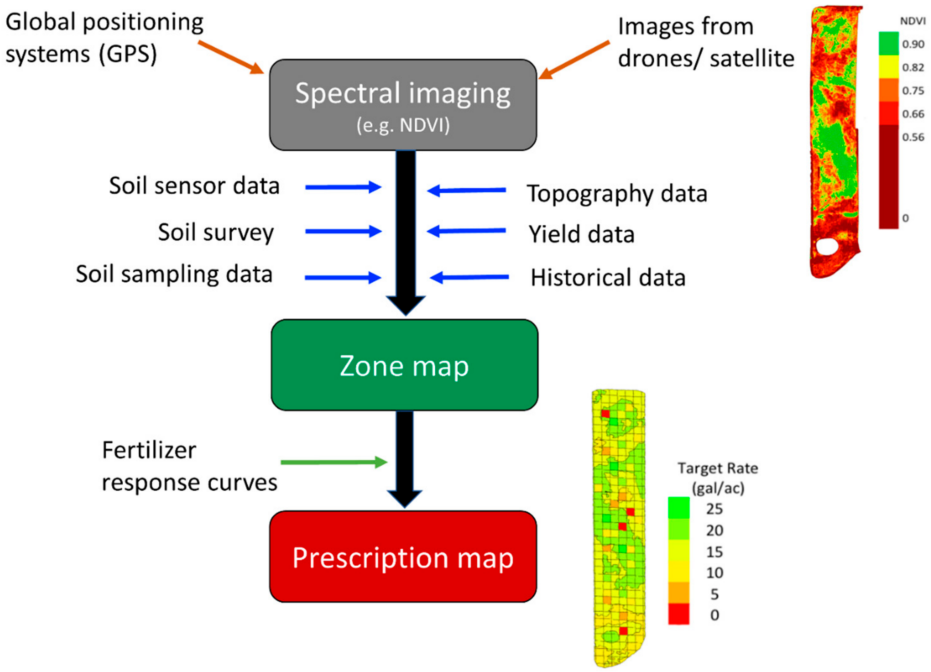

Figure 2. A conceptual model showing how prescription maps can be generated for field application of symbiotic nitrogen fixation (SNF)-optimizing inputs. GPS-enabled spectral images (e.g., NDVI maps) can be generated by different software programs, by using in-season aerial imagery captured from satellite/unmanned aerial systems. Then, spectral images can be used to generate management zones (zone creation) within a given field, by integrating yield data, soil data (e.g., data from sensors, surveys, soil sample analysis), topography data, and other available historical data (e.g., cropping history). Finally, zonal maps can be used to generate the prescription maps by considering the fertilizer response curves (e.g., N response curve) of different crops.

It is important to note that not all the N fixed by legumes is retained within the plant or harvest. N fixed by legumes can also be released from root systems during the growing season (mainly as root exudates) [8,114] or during the subsequent growing season (primarily due to decomposition) [7]. Organic N release helps to improve soil N availability for subsequent crops and promotes N transfer to neighboring non-legumes in a mixed/intercropped field. However, fixed N released from legumes can contribute to nitrate leaching [115]. Available soil N and leachates can be measured using in-field sensors or lysimeter monitoring systems [115,116]. Therefore, combining all these inputs, it is envisioned that future variable rate prescription maps will optimize not only input supplies relevant for SNF (e.g., P, Mo) but also minimize N losses from SNF (e.g., nitrate leaching) within a given field, to achieve sustainable legume-based production systems.

Author Contributions: Both M.S.T. and M.N.R. conceived of the manuscript. M.S.T. analyzed the literature and wrote the manuscript, and M.N.R. edited the manuscript. Both authors read and approved the final manuscript.

Acknowledgments: We thank Jacob Nederend and Deveron UAS Corp. for giving us permission to use their NDVI images in Figure 2. This study was supported by CIFSRF Grant 107791 to Manish Raizada from the International Food Development Centre (IDRC, Ottawa) and Global Affairs Canada.

Conflicts of Interest: The authors declare no conflict of interest.

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