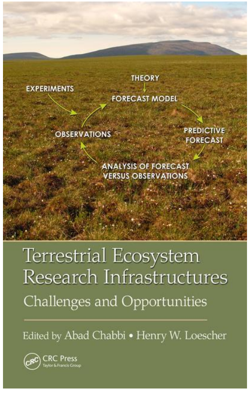

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Challenges and Opportunities
Abad Chabbi, Henry W. Loescher

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Field Phenotyping: Concepts and Examples to Quantify Dynamic Plant Traits across Scales in the Field

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CONTENTS

Abstract

The increase in population is leading to an unprecedented demand on agriculture and natural resources. To meet human and nature needs, food production must drastically increase while, at the same time, agriculture's environmental footprint must decrease dramatically. In times of global change, a better understanding of the dynamic spatiotemporal adaptation of our crops is needed to provide the basis for crop breeding, management, and protection measures. To scientifically understand the mechanisms behind the dynamic structural and functional adaptation of plant traits, it is mandatory to phenotype plants under natural, that is, fluctuating environmental conditions in the field. Plant phenotyping aims for a quantitative description of plant traits, which is affected by genetic variation as well as by the environment.

In this chapter, we review and develop concepts for phenotyping of dynamic plant traits in the field. We give an overview on the most prominent sensors and measurement concepts that are the backbone for plant phenotyping initiatives. We focus on the quantification of most relevant traits, which are related to functional and structural root development, plant structure, water relations and transpiration, and photosynthetic energy conversion. In addition to the sensors and measurement modes, we review positioning systems that allow for a reproducible recording of experimental plots at different temporal and spatial resolution. Finally, the quantitative data on the plant's phenotype is integrated with environmental data and information on anthropogenic impact to better understand gene × environment × management interactions, which ultimately will be the basis for a sustainable and resource-efficient use of our plant resources in a future bio-based economy.

Keywords: Plant phenotyping, Root, LIDAR, Stereo cameras, Thermal imaging, Active thermography, Hyperspectral, RGB camera, Vegetation indices, Chlorophyll fluorescence, Ground positioning systems, UAV, Airplanes, Environmental monitoring

3.1 Introduction

Humankind has already entered an era where anthropogenic interference dominates large parts of our planet. The growing human population and high living standards are the driving forces for increasing demands on our Earth's ecosystem services, which in turn are leading to a greatly increased use of marine and terrestrial ecosystems. As a consequence, we are now facing four main global challenges:

1. Safeguard food, feed, and water supply to a growing global population
2. Ensure the availability and access to energy for everyone
3. Protect and sustain natural resources and the environment
4. Ensure human well-being and health to the world population

Addressing any one of these major challenges cannot be considered as a stand-alone task because of their interconnectedness. To increase food production, more land and energy may be required, thus, exacerbating already-existing pressures on nonrenewable resources. To satisfy an increasing demand for renewable energy, we may need to enhance biomass production for biofuels, thus competing for agricultural land required for food production. To protect the environment and its important services and to preserve biodiversity, we must avoid agricultural intensification potentially affecting food production.

Sustainable agriculture and primary vegetation productivity are the basis for a future bio-economy. The EU strategy and action plan named bio-economy as a key element for smart and green growth in Europe that reconciles food security with the sustainable use of renewable resources for industrial purposes, while ensuring environmental protection (McCormick and Kautto 2013).

But earth's primary productivity is constantly under the threat of several external abiotic and biotic factors, such as drought, extreme temperatures, pests, and nutrient limitation. The magnitude and impact of some of these factors may be exacerbated by global changes, which involve climate, but also major changes that are caused by a rapidly growing human population and the current unsustainable utilization of some critical, but limited and nonrenewable resources.

Thus, the sustainable and resource-efficient use of our vegetation is of outmost importance for the survival of humankind. Great advances have been made in our understanding of the potential of the genetic background of our vegetation and many agricultural crops like rice (Sequencing Project 2005) or recently wheat (Eversole et al. 2014) are sequenced. After these major steps forward, nowadays the plant phenotype is put in the center of plant research. We need to identify which genes are expressed under which environmental and management conditions (i.e., drought, extreme temperatures, pests, and nutrient limitation) resulting in a certain phenotype.

On the other hand, plant breeders, ecosystem ecologists, and agronomists need accurate and reliable sensing technologies to support their strategic decisions by detailed spatial and temporal information on plant growth (and plant growth stages) to predict future plant development and yield, as well as ecosystem functions, for example, net primary productivity and water use (Auernhammer 2001). These methods must be able to bridge spatial areas (e.g., from single plants to experimental plots, fields, and ecosystems) to deliver the necessary data to the breeder, land manager, or scientist. One key problem under field conditions is the large number of plant phenotypes as a result of almost endless permutations of genetic information and environmental conditions.

Plant phenotyping in the strict sense is not a fully new discipline. For decades agronomists and plant scientist have described the phenotype of a plant in the greenhouse and in the field. Optical approaches to detect crop status have already been developed in the 1950s and 1970s (without actually naming these approaches plant phenotyping) (Moran et al. 2003). Also, noninvasive measurements were often the method of choice to reproducibly and repeatedly quantify plant traits under greenhouse and field conditions.

Considering the recent technological developments, there are currently several phenotyping initiatives that aim to set up and improve plant phenotyping in the laboratory and also under field conditions. It is generally agreed that the discipline needs both controlled laboratory and greenhouse approaches as well as field approaches, where the plant is exposed to natural and thus varying environmental conditions (Rascher et al. 2009). Especially in the field, the environmental conditions vary by nature, and the plant phenotype has to be characterized under these nonstationary conditions, which adds an additional dimension on the requirement for sensors and the recording of time series with the appropriate spatial and temporal resolution to address salient questions. In this context, the long-term ecological research field sites (LTER), the Carbo Europe long-term study sites, various institutional field sites, and decades of ecophysiological plant research have provided the basis for modern field phenotyping activities. Knowledge from these interdisciplinary projects is the basis for sensor development, measurement protocols, and the scientific understanding of dynamic adaptation of plant traits.

The need for improved technical and scientific demands has resulted in various integrated activities worldwide, where, on the one hand, research institutions, agencies, and universities have developed phenotyping infrastructure and, on the other hand, user networks are developing the scientific tools, knowledge, and practical applications.

In this chapter, we will review the four main pillars that are needed to develop a network of plant phenotyping infrastructure. To address the challenges mentioned earlier, we will need to identify

- Which plant traits need to be monitored to understand the dynamics of the gene × environment × management interaction that determines the expression of a plant's phenotype
- A set of sensors and measurement approaches that allow fast and noninvasive quantification of relevant plant traits

- A set of positioning systems that facilitate to place the sensors in an automated and reproducible manner in the field
- A concept for environmental sensors to link the plant phenotype to the dynamic changing environment

3.2 Concept of Field Phenotyping: From Traits to Sensors and Positioning Systems

3.2.1 Relevant Traits Define Sensors for Field Prototyping

To understand how changes of environmental conditions will modulate the expression of crop genetic information, we need first to identify the relevant traits that need to be monitored. For field phenotyping, we propose four main clusters: (1) structural and functional root traits, (2) canopy and plant developmental morphology, (3) traits that determine water relations (e.g., water uptake and transpiration and water-use efficiency) of plants, and (4) functional traits that are related to photosynthetic energy conversion and carbon uptake (Figure 3.1). Depending on the scientific question of interest, those traits need to be monitored on different scales ranging from leaf, whole plant to the canopy scale. Only a good understanding of the dynamic changes at leaf, plant, and canopy level will allow us to understand field-scale

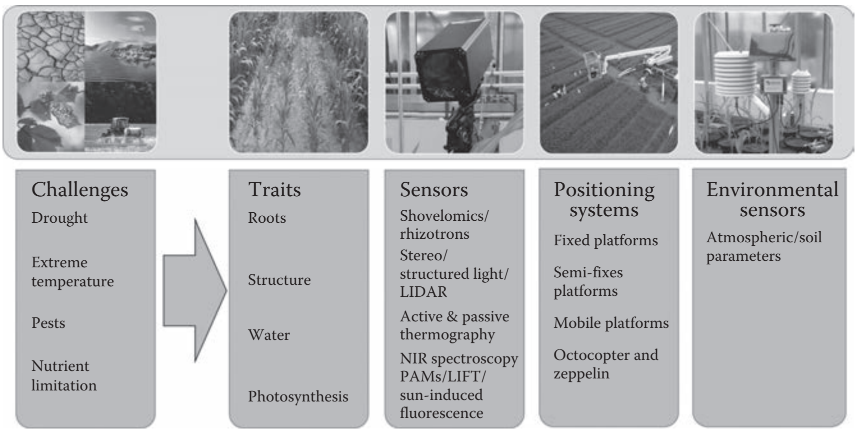

FIGURE 3.1
The pillars of field phenotyping. To evaluate relevant traits, the right sensors have to be chosen and they have to be positioned by dedicated field positioning systems. This infrastructure then can be applied in dedicated field experiments that are additionally equipped with dedicated environmental sensors.

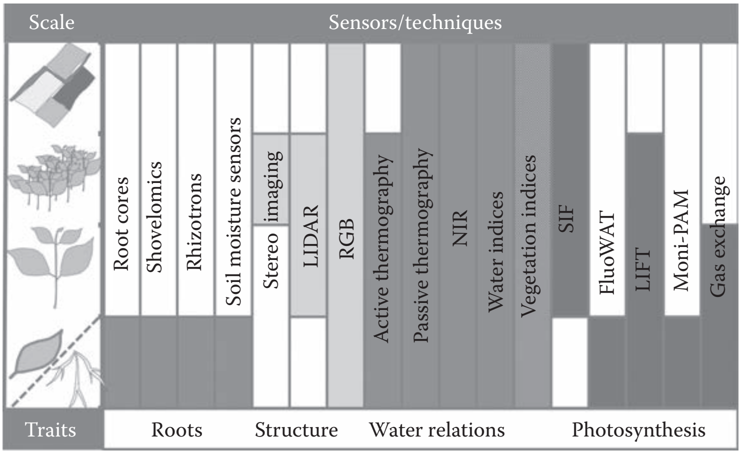

FIGURE 3.2
(**See color insert.**) Scheme of the sensors and techniques to characterize plant phenotypic traits for roots, structure, water relations, and photosynthesis, where the colored part of the column indicates the scale they can be employed for. These are (1) roots mostly by excavation, (2) individual leaves, for example, by clip on devices, (3) capturing whole plants or measuring organs within canopy, (4) on top of canopy mostly by mobile platforms, and (5) field from flying platforms and satellite.

measurements. In the framework of several projects, promising sensors were identified and measurement concepts to quantify traits were developed to analyze plant structure (root and shoot), photosynthesis, and water relations at different scales (Figure 3.2).

3.2.1.1 Measuring Root Traits

Many of the traits required in future crops are tightly linked to root properties and root system architecture which can strongly affect crop yield. The root system is very plastic and responds strongly to environmental influences. Sustainable plant production rely on root systems adapted to growing conditions in the field.

In the past, the majority of root phenotyping efforts were focused on establishing phenotyping platform in the green house, where the gene × environment effects are often missing (Kuijken et al. 2015). The main reason for this negligence is the technical difficulty in accessing the roots for phenotyping in the field, especially when temporal and spatial variability have to be considered too. Roots can be measured directly or indirectly in the field using destructive and nondestructive phenotyping technologies. Direct, destructive phenotyping methods are made by digging the roots out or exposing them in one point and time, such as manual coring or via shovel (Rich 1990; Trachsel et al. 2010). Both coring and "shovelomics" can be carried out rapidly to achieve the required numbers and repetition needed for field phenotyping to compare genotypes (Wasson et al. 2014; York and Lynch 2015). However, both approaches are not dynamic because they cannot be repeated over time on the same root systems.

A true dynamic approach is the use of rhizotrons, where repeated measurements of the same root systems are possible. "Mini-rhizotrons" are cylindrical clear tubes, inserted into the ground so that roots that grow on the tubes can be imaged by a camera within the tube. For instance, mini-rhizotrons have been used to measure the time course of descending root profiles of different crop species (Thorup-Kristensen and van de Boogaard 1998). To fully understand root allocation patterns, a traceable and reproducible approach to quantify the camera's technical imprecision is a prerequisite to avoid the risk of performing an improper quantification of the root system architectures (Roberti et al. 2014).

Unless the crop is very young, all technologies described earlier have the common problem of sampling a very small portion of the root systems. Coring only takes a small vertical transect through a mixture of roots below the crop canopy and generally the cores are of small diameters (4–10 cm). Shovelomics takes a single root system to a depth of 20 or 30 cm, leaving behind roots torn away during the shoveling and washing process. Mini-rhizotrons provide a small viewing window (cm range), having the added disadvantage of requiring insertion prior or during crop establishment.

Root phenotyping in the field is on a very early stage and reliable noninvasive methods to quantify root traits on the appropriate spatial and temporal level are still not available. Thus, much work is needed to develop the appropriate measurement concepts to improve root phenotyping in the field.

3.2.1.2 Measuring Structural Traits

Plant structure and function are known to be linked in natural and agronomic systems like leaf angle distribution, that in corn greatly influences light interception and yield in densely planted canopies (Ford et al. 2008). During their seasonal development, most plants display strong morphological changes, which depend on the availability of resources and on the fluctuation of abiotic and biotic factors. For instance, leaf orientation can be greatly affected by environmental factors like drought, which can be highly useful for breeders to compare drought stress tolerance between cultivars (Müller-Linow et al. 2015). Structural properties that are altered on the diurnal and seasonal scale may affect the efficiency of light interception within the canopy and thus may influence canopy light-use efficiency (Müller-Linow et al. 2015).

The most commonly used geometric measure of plant canopy is the leaf area index (LAI), which relates the projected leaf surface to the soil surface. However, the LAI does not provide information on plant architecture or the distribution of leaf orientations. For nonmanual estimation of plant architecture, several methodical approaches are available, which include structured light approaches, laser scanning techniques, and stereo imaging. In structured light techniques, the reconstruction of a plant's shape is derived by analyzing the distortion of a projected light pattern (Kjaer and Ottosen 2015). High-resolution 3D models can be achieved, which often also allow to separate objects having different colors (e.g., fruits and leaves) (Bellasio et al. 2012). Stable light conditions are needed to perform structured light measurements thus it is limited to greenhouse or growth chamber environments.

Alternatively, LIght Detection And Ranging (LIDAR) systems can measure the distance between the sensor and the objects around it very fast, enabling the construction of 3D point clouds. Appropriate algorithms can transform these point clouds into a highly precise digital reconstruction of crop structures and architecture (Sanz-Cortiella et al. 2011).

In recent years, stereo camera setups were developed (Biskup et al. 2007; Müller-Linow et al. 2015). Two cameras with a defined baseline distance are used to record simultaneously stereo images. From these stereo images, the distance of canopy elements can be computed and a full 3D representation of the canopy can be calculated. Further processing of the data can reveal relevant structural parameters such as canopy area, leaf angle distribution, the number of leaves, or the ratio between leaves and fruits, which translates impressive 3D images in quantitative data on vegetation properties (Rascher et al. 2010; Müller-Linow et al. 2015; Figure 3.3).

Dynamic changes in canopy structure were long unvalued in science and only limited implications of structural variations on ecosystem functioning were assumed. In recent years, however, structure–function relations have recently being put in the focus of breeding strategies as structural optimization may still pose some potential for yield improvements (Zhu et al. 2010). During growth, light availability becomes increasingly limited and fluctuating in the lower canopy layers. Plants may adapt to this by layer-specific distributions of leaf orientations. Structural changes are recognized to sensitively display stresses such as leaf rolling when plants are exposed to drought stress (O'Toole and Cruz 1980). Elevated atmospheric CO_2 concentrations were described to increase the total leaf area in soybean shoots (Ainsworth et al. 2002), and wind logging has been put back on the agenda of breeders to reduce the economic loss produced by wind. Thus, the quantitative understanding of the interplay of structural canopy traits with their environmental conditions is currently revisited, even though one does not want to underestimate the complexity, which is behind the mechanisms that control canopy structure.

3.2.1.3 Measuring Water Relations Traits

Breeding for drought tolerance has to consider various traits responsible for plant water status. Leaf water potential may be the most important parameter, but it is impossible to measure water potential nondestructively. Thus, various other methods have been tested that assess either leaf and canopy

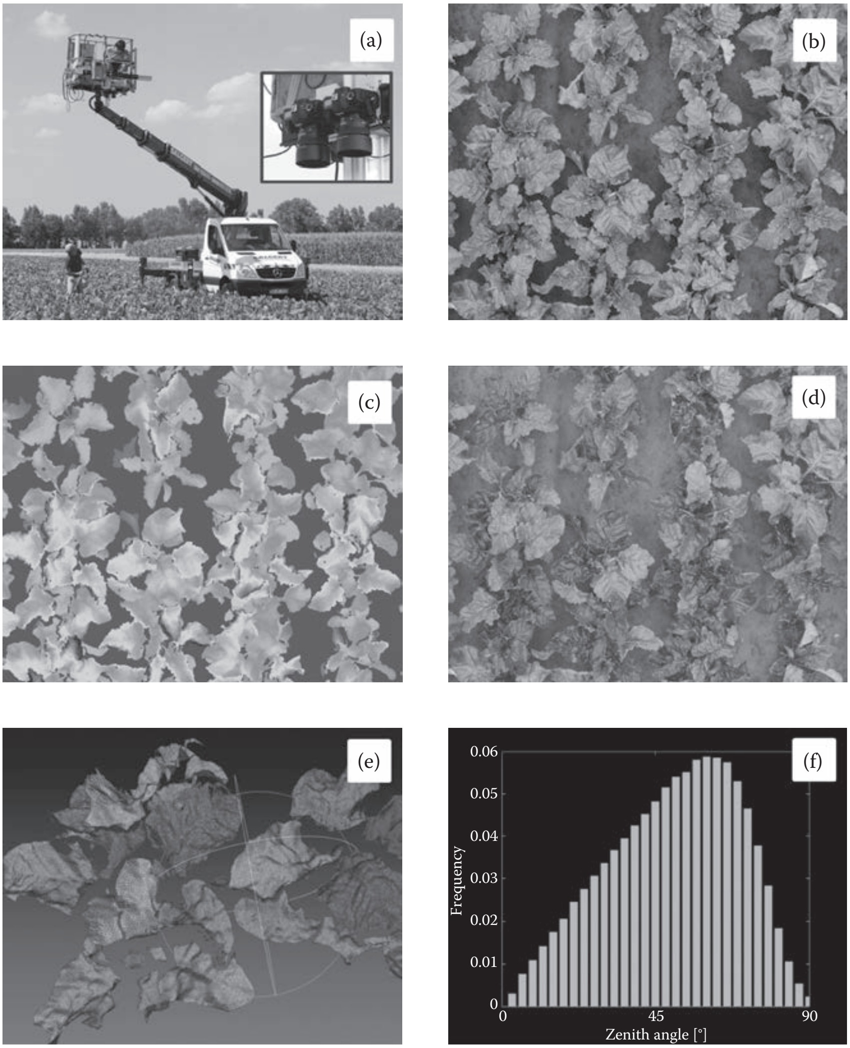

FIGURE 3.3
(**See color insert.**) Estimation of the leaf angle distribution from a 3D reconstruction of a sugar beet canopy: (a) The stereo camera setup (see insert) has been mounted on a cherrypicker 3.5 m top of canopy; (b) shows a region of interest with ~6 plants in the original left camera RGB; (c) filtered depth (disparity) map of (b) with pixel colors indicating the object depth; (d) single leaf segmentation, which is used for further individual leaf surface modeling; (e) leaf surface models are used to derive different leaf traits like leaf area, canopy area, or leaf angle distributions; (f) the leaf angle distribution of the zenith angle of the previous reconstruction; the zenith angle ranges from 0° (flat leaf surface) to 90° (errected surface) and can derived locally or for the complete leaf.

water content or approximate transpiration rates (Farrar et al. 2011; Sampoux et al. 2011). In this context, measurements of near-infrared (NIR) and thermal imaging have been introduced as the most promising measurement approaches in the lab and in the field (Fiorani and Schurr 2013).

A qualitative approach to estimate plant water content is NIR measurements using the relative depth of the water absorption band in the NIR region (1370 and 1870 nm). This approach generally provides a good relative estimate of water content but to our knowledge there is no study available that describes the retrieval of plant water content as absolute physical values.

A second approach is exploiting thermal cameras, which are sensitive within the infrared region (9–13 μm spectral range) to evaluate plant transpiration and evapotranspiration (ET). The principle of passive thermography is that surfaces are cooled by ET, so surface temperatures are lower than ambient temperature, which is proportional to the rate of ET. However, leaf temperature (T_L) does not only depend on ET rates but also depend on the leaf boundary layer, which is a thin layer of air at the leaf surface. The thickness and composition of the leaf boundary layer determine how fast heat can be dissipated, that is, increasing leaf boundary layer decreases the transfer of heat from the leaf to the atmosphere and vice versa (Leuning et al. 1989). Furthermore, leaf boundary layer and thus T_L respond in a dynamic way to variable environmental conditions. Parameters such as solar irradiance and ambient air temperature are highly fluctuating and highly affect the leaf boundary layer.

To overcome these problems, Jackson et al. (1981) developed the crop water stress index (CWSI), which normalizes leaf temperature against the prevailing environmental conditions. This index is based on the comparison of leaf temperature to wet and dry reference surfaces. The CWSI and other indices have been shown to be sensitive to evapotranspiration and can be used to detect drought stress–induced stomatal conductance (Jackson et al. 1981; Jones 1999; Cohen et al. 2005; Grant et al. 2006; Möller et al. 2007; Alchanatis et al. 2009).

Passive thermography, and particularly the CWSI, has become a widely used tool for measuring plant evapotranspiration to analyze high numbers of plants in a short period of time. Passive thermography measures only one part of the overall plant–water relations, namely, ET. To understand plant–water relations in whole plants in response to changing environmental conditions (e.g., drought), it is essential to know how different water fluxes between roots, stems, and leaves are connected.

A promising approach is active thermography, where T_L is actively manipulated by a short heat pulse. An additional heat pulse increases T_L transiently. After a short time, T_L will decrease again approaching the former steady state. The time constants (τ) of heating or cooling can be measured. This time constant (τ) depends on the leaf heat capacity per unit area (CA_{leaf}^{-1}) and the leaf heat transfer coefficient (h_{leaf}). High leaf water content leads to a higher heat capacity and consequently higher τ. The second parameter affecting τ is h_{leaf}, which describes how fast a leaf is able to dissipate heat. h_{leaf} depends on the boundary layer and is therefore highly affected by environmental conditions. For instance, increasing wind decreases the boundary layer, which in turn increases h_{leaf} and decreases τ. Also, stomatal conductance affects h_{leaf}. High stomatal conductance accelerates leaf heat dissipation and consequently h_{leaf} increases and τ decreases. Active thermography was successfully tested in laboratory at leaf scale and under greenhouse conditions at canopy scale. At leaf scale, a linear relationship between τ and LWC and consequently CA_{leaf}^{-1} was found. This relationship changed when the leaf boundary layer and thus h_{leaf} was manipulated by wind.

We would like to highlight the need to combine passive and active measurements to fully understand the overall plant–water relations in response to drought. Intuitively, the water content limits water loss by transpiration and thus controls transpiration rates. However, neither the CWSI alone, nor τ alone are appropriate to reflect this relation. By combining τ with CWSI, one may be able to track changes in LWC, boundary layer conductance, and transpiration. This will facilitate a better understanding of the dynamic responses of plants to optimize their water relations and help to better understand the strategies to cope with drought stress.

3.2.1.4 Measuring Photosynthesis Traits

Plant growth and productivity do not only depend on the availability of nutrients and water but to a great extent on adequate sunlight and temperature. Variations of these conditions affect photosynthesis rates and consequently plant productivity. Even though photosynthesis being the primary process that determines plant growth, there is no simple link between photosynthetic CO_2 uptake rates and plant growth (Körner 2000). Nevertheless, measurements of photosynthesis are essential to either refine plant growth models or alternatively photosynthesis may be used as a sensitive indicator for environmental limitations of a plant energy metabolism.

In the past decades, different approaches have been used to estimate photosynthesis, including gas exchange measurements, spectral vegetation indices, and chlorophyll fluorescence.

Gas exchange measurements are widely used at leaf and plant level. The portable infrared gas analyzers using small leaf clip chambers allow measurements at leaf level, whereas customized whole plant chambers are built in the field to measure at canopy level (Burkart et al. 2007). These methods however are very labor intensive and may never be used for rapid screening of numerous field plots. They are nevertheless essential tools for method validation discussed in the following text, which allow noninvasive measurements of photosynthesis at field plot scale.

VIS-NIR spectroscopy has become a versatile and accessible proxy for plant photosynthetic capacity. Spectral analysis can be performed at all different scales using point and imaging spectroscopy (Chuvieco and Huete 2010). The addition of spatial information by imaging spectroscopy offers new opportunities for plant phenotyping. A high number of so-called spectral vegetation indices (VIs) have been developed to quantify pigment contents as well as structural and physiological properties at leaf, plant, and canopy level (Jackson and Huete 1991). VIs combine spectral information of two or more bands. The selected spectral bands are combined in a manner that enhances the reflectance properties of specific molecules or that could identify plant stress response (see Jansen et al. 2014 for an overview on the most commonly used VIs). Mainly due to its simplicity, VIs are widely used in research in the area of breeding, precision agriculture, and remote sensing. Nevertheless, one should not miss the potential of using the full vegetation reflectance spectra for plant phenotyping. Some of the current and most powerful approaches are partial least squares regression (Feilhauer et al. 2010), supervised and unsupervised endmember selection and unmixing, continuous support vector machines (Hostert et al. 2003), multi-block analysis (Eiden et al. 2007), or simplex volume maximization (Roemer et al. 2012). All these methods provide significantly more accurate results than the use of VIs; however, their application for phenotyping purposes requires major adaptation of computer algorithms and data processing.

However, the reflectance-based approaches only allow a quantification of photosynthesis pigments. Functional regulation of the photosynthetic apparatus may be reflected in absorption changes around 531 nm that are related to the epoxidation of the xanthophyll cycle pigments during non-photochemical energy dissipation. These changes can be measured using the photochemical reflectance index (PRI), which is widely used in phenotyping and remote sensing (Gamon et al. 1990; Garbulsky et al. 2011). It has to be noted that the PRI is greatly influenced by structural factors, such as chlorophyll/carotenoid ratio, leaf angle, and illumination geometry (Barton and North 2001), and thus this index has to be used with care.

Currently, the most widely used technique to quantify the efficiency of photosynthesis exploits the fluorescence signal of chlorophyll. Chlorophyll fluorescence is light reemission in the red (690 nm) to NIR (740 nm) following light absorption by photosynthetic pigments (chlorophylls and carotenoids) in plants. The principle underlying the use of chlorophyll fluorescence as an indicator of plant photosynthetic status is relatively straightforward. Absorbed light energy excites chlorophyll molecules and de-excitation of this energy is mainly attained through three competing processes: photosynthesis, radiative loss of photons or chlorophyll fluorescence, and non-radiative thermal energy dissipation (non-photochemical quenching, NPQ). As these three energy dissipation processes compete for excitation energy, changes in one process (e.g., photosynthesis) will affect the other two. Hence, by measuring chlorophyll fluorescence, we can derive information on NPQ and photosynthesis (Maxwell and Johnson 2000; Porcar-Castell et al. 2014). Major developments in the instrumentation for measuring chlorophyll fluorescence have been made in the last decade, and currently more than 500 scientific articles are published each year that use this method* (for recent reviews, we refer to Baker 2008, Papageorgiou and Govindjee 2004). The most widely used technique is the pulse-amplitude modulation (PAM); in this approach, chlorophyll molecules are brought to excited states by absorption of active measurement light (Schreiber 1986, 2004). The handheld Mini-PAM device allows for a quick assessment of leaf fluorescence, whereas the stationary Monitoring-PAM (e.g., Porcar-Castell et al. 2008) is developed for field measurements and can be clamped on the leaf permanently as long as weather conditions or leaf growth allow. Upscaling this method to measure the canopy from above is limited by the close vicinity needed to apply a saturating light pulse. In recent years, the light-induced fluorescence transient method (LIFT, Kolber et al. 2005; Pieruschka et al. 2010) was developed. This method allows measurements of fluorescence parameters from a distance of several meters. This so-called pump-and-probe technique has been developed and further improved for applications in the field (Kolber et al. 2005; Rascher and Pieruschka 2008; Pieruschka et al. 2014; Raesch et al. 2014).

For large-scale studies, active methods will not be applicable and thus alternative methods to passively quantify the fluorescence emission have been developed. Such passive techniques retrieve chlorophyll fluorescence emission from the solar irradiance and the vegetation-emitted radiance by using the absorption bands in surface solar irradiance (termed sun-induced fluorescence). The most important atmospheric absorption bands in the fluorescence emission region are two of the oxygen absorption bands: O_2-A at 761 nm and O_2-B at 687 nm. The Fraunhofer Line Discrimination (FLD) principle (Plascyk 1975; Plascyk and Grabriel 1975) allows to retrieve chlorophyll fluorescence emission in these absorption lines (see Meroni et al. 2009 for an in-depth review). This technique allows estimation of absolute variations in the sun-induced fluorescence intensity from leaf up to regional scales. At leaf scale, the FluoWat leaf clip (Alonso et al. 2007; Van Wittenberghe et al. 2013) has been developed to measure the whole chlorophyll fluorescence emission spectrum by clip-on approach. At canopy scale, high-resolution point spectrometers can be used to retrieve sun-induced fluorescence emission in the oxygen absorption lines (Rossini et al. 2010; Burkart et al. 2015). Today, state-of-the-art imaging spectrometers can be used to map the spatial distribution of the sun-induced fluorescence signal in the wider atmospheric oxygen absorption lines, which provides first insight into the spatiotemporal dynamics of fluorescence emission in natural canopies (Pinto et al. 2016).

An important advantage of sun-induced fluorescence is the possibility to be scaled up to larger areas. As it relies on passive detection of the emitted

* Based on a bibliographic survey in Web of Science (core collection), the following number of papers were published using the keywords "photosynthesis" *and* "fluorescence": 2011: 537 publications; 2012: 595 publications; 2013: 609 publications; 2014: 724 publications; 2015: 642+ publications.

fluorescence, this method can also be used with unmanned aerial vehicles (UAVs, e.g., multicopter), aircrafts, and even satellites. There is currently no reliable sensor for drones available yet, but one can expect that such miniaturized sensors will become available soon. Recently, an airborne sensor was released that allows the exact quantification of sun-induced fluorescence from a research aircraft (Rascher et al. 2015; Rossini et al. 2015). This sensor, called *HyPlant*, was proven to deliver novel information of large field trials. Figure 3.4 shows an exemplary *HyPlant* flight line covering a large agricultural area close to the Forschungszentrum Jülich (adapted from Rascher et al. 2015).

On the largest scale, recently the European Space Agency (ESA) selected the FLuorescence EXplorer (FLEX) mission to measure sun-induced fluorescence on a global scale in their new Earth Explorer 8.* Thus, we expect great progress in this method over the next years that will derive from the possibility to globally map vegetation health and stress.

3.2.2 Sensor Positioning Systems: A Compromise between Temporal and Spatial Resolution

To survey large fields, devices and sensors are required that deliver detailed and reproducible temporal and spatial information on the cultivated crop phenotypes on a regular base. In the following sections, we will describe and provide examples for the different positioning systems, from ground-based systems to airplanes and satellite platforms.

3.2.2.1 Ground-Based Systems

Ground-based system can be divided into four different groups: (1) handheld systems, (2) fixed platforms, (3) semi-fixed platforms, and (4) mobile platforms (Table 3.1). The main differences between these ground-based platforms are found in their compromise in temporal and spatial resolution. In other words, platforms enable either manual or semi-manual measurements with limited reproducibility or automated measurements with high reproducibility. Handheld sensors can be carried by a single person through the field, but they are excessively labor intensive and time consuming; they are not useful for high-throughput phenotyping. Fixed platforms are permanent infrastructures built over a specific field which is able to position different sensors from one experimental plot to another and offer a high flexibility regarding temporal and spatial resolution. The main drawback of this approach is that the platform movement is limited to a single field. Semi-fixed platforms, like towers, elevated platforms, or "cherry pickers," allow performing measurements in a high temporal resolution over extensive areas, which simplifies the comparison of different experimental

* http://www.esa.int/Our_Activities/Observing_the_Earth/New_satellite_to_measure_plant_health.

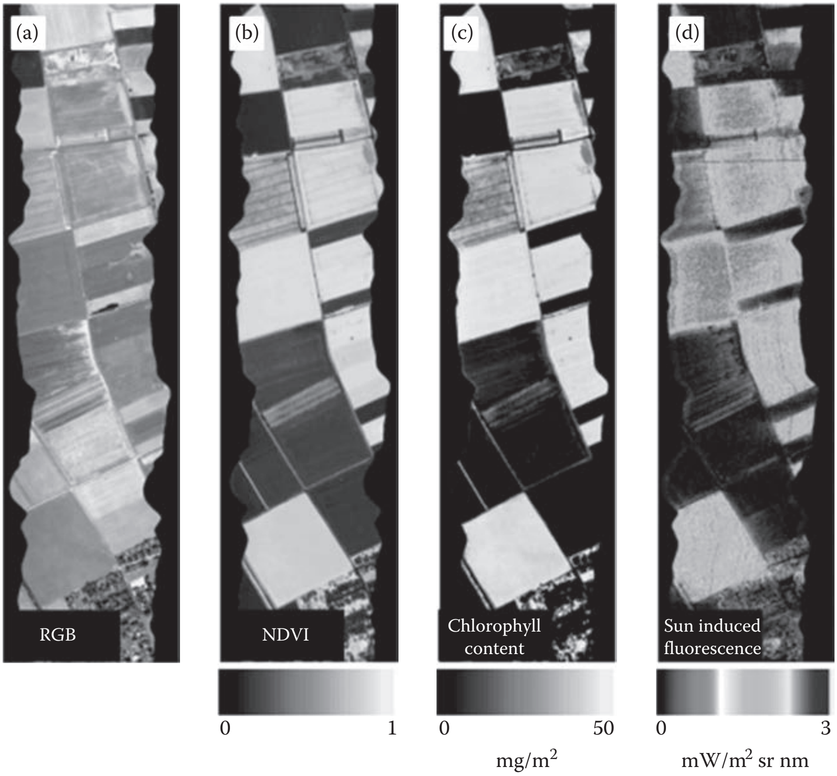

FIGURE 3.4
(**See color insert.**) Airborne maps of different vegetation products and sun-induced fluorescence (SIF) from an agricultural area in Western Germany. The flight line was recorded on August 23, 2012, from 600 m height, at 13:50 local time (UTC + 2 h), which was approximately 1 h after solar noon. (a) For the pseudo-RGB image, reflectance bands at 696, 708, and 677 nm are used for the red, green, and blue channel of the image. (b) The normalized difference vegetation index (NDVI) was calculated as NDVI = R758 – R670R758 + R670. (c) Leaf chlorophyll content was calculated using the Gaussian process regression method (Verrelst et al. 2012, 2013). (d) Sun-induced fluorescence (F760) was calculated using the 3FLD method with an empirical correction of effective transmittance in the relevant wavebands. Dominant green vegetation in this area at the time of observation were sugar beet and corn on one side and grassland on the other side. The two vegetation types were in contrasting periods of their seasonal development. Sugar beet and corn had a dense fully mature canopy in contrast grassland had reached senescence. A visual evaluation already indicates that fluorescence shows a different pattern compared to other vegetation variables. This indicated the added value of the sun-induced fluorescence signal that provides a new window into photosynthetic functioning that potentially can be mapped on large areas.

TABLE 3.1

Ground-Based Phenotyping Platforms

	Example	Temporal and Spatial Resolution	Disadvantages	Advantages
Handheld sensors can be carried through the field by a single person	Point spectroradiometers Thermal sensors Imagers	Low	Labor intensive and time consuming	Mostly used as a ground true reference to validate measurements performed from UAVs and airplanes
Fixed platforms permanent structures built over a specific field which are able to move different sensors from one experimental plot to another	Field Scanalyzer, Lemnatec[a] Eidgenössische Technische Hochschule, Zürich's Field Phenotyping Platform[b]	High	Limited to a single field	Possible to carry a broad number of sensors
Semi-fixed platforms towers, elevated platforms or "cherry pickers"	FieldLIFT, Forschungszentrum Jülich[c]	High	Plots further away are both smaller and have more atmosphere to traverse (relevant for both thermal and reflectance data). Difficult to move	Possible to carry a broad number of sensors

(*Continued*)

TABLE 3.1 (***Continued***)

Ground-Based Phenotyping Platforms

	Example	Temporal and Spatial Resolution	Disadvantages	Advantages
Mobile platforms simple hand-pushed bicycle to sophisticated platforms which can traverse the field autonomously or with a driver	Field4Cycle, Forschungszentrum Jülich[c] FieldCOP, Forschungszentrum Jülich[c] BreedVision, Osnabrucke, Germany (Busemeyer et al. 2013) Avignon system, France (Comar et al. 2012) Phenomobile Canberra, Australia (Deery et al. 2014)	High	Data handling, tons of gigabytes that need to be first processed and then combined together to generate effective measurements for a particular experimental plot	Possible to carry a broad number of sensors Constant view angle FieldCOP, work independently including night measurements

Source: Adapted from Deery, D. et al., *Agronomy*, 4(3), 349, 2014.

[a] http://www.lemnatec.com/.

[b] http://www.kp.ethz.ch/infrastructure/FIP.html.

[c] http://www.fz-juelich.de/ibg/ibg-2/EN/methods_jppc/methods_node.html.

plots. Mobile platforms ranging from simple hand-pushed bicycle system to sophisticated platforms which can traverse the field autonomously or by a driver offer the possibility to perform measurements in a high temporal and spatial resolution over extensive areas. Data handling is one of the major challenges of using mobile platforms. High temporal and spatial resolution means tons of gigabytes of data from different sensors (frame imagers, line-scan imagers, and point sensors) with their own spatial resolution. These data need to be processed first and then combined to generate effective measurements for a particular experimental plot.

3.2.2.2 UAVs and Unmanned Aircrafts

The recent developments in UAVs provide platforms for positioning lightweight sensors over agricultural fields. The UAV as a free movable platform is a "very versatile tripod" that enables the positioning of a sensor almost everywhere over agricultural fields. UAVs are capable of providing airborne data with a high spatial and temporal resolution due to their flexible and easy use. This opens up new possibilities in precision farming and management or huge breeding experiments. While common RGB cameras already provide in-depth insights, if positioned above agricultural fields (Sakamoto et al. 2012; Bendig et al. 2014), other sensor types allow complementing analyses. Recent technical developments toward small high-performance electronics allowed the production of thermal cameras that can be carried by UAVs to detect water stress (Zarco-Tejada et al., 2012). Just recently highly accurate and lightweight point spectrometers and multispectral cameras became available, which make them suitable for the use in UAVs (Burkart et al. 2014).

The retrieval of small differences in the reflectance of plants is necessary to detect relevant differences in traits. But to identify these small differences, the quality of the measurements must allow such discrimination. However, field measurements with lightweight sensors that are often used on UAVs are prone to a large variety of undesired influences increasing uncertainty. The reflection signal of plants can be altered by these uncertainties arising from variable environmental factors, such as changes in light conditions by clouds, different sun elevations, variation in temperature, or strong wind. Additionally, data retrieval depends on technical characteristics and the quality and stability of the sensor itself. Thus, to not only produce colorful UAV-based pictures, specific measurement reports have to be adapted for the combination of environmental factors and instrument characteristics to insure consistent data quality.

An alternative to the use of UAV for field phenotyping is the use of zeppelins. The high payload, extended flight time, smooth flight, and low vibrations make the airship a great platform even for sensitive scientific instruments, complementing other small UAV platforms.

3.2.2.3 Airplanes and Satellites for Field Phenotyping

Most of the sensors and measurement approaches that were described earlier are applied on the plot scale. This is the most relevant scale from a scientific perspective and for breeders and agricultural management. However, field phenotyping will be expanded to a larger scale in the near future. Demand comes not only from precision agriculture but also from research in agroecology (Diekötter et al. 2014).

In this context, aircraft- and satellite-based approaches are becoming increasingly important. On these large-scale passive measurements, new methods are becoming favorable. Current methods that can also be used with air- and spaceborne sensors include 3D surface reconstruction using LIDAR and stereo camera approaches (Asner et al. 2012), thermography (Munns et al. 2010), imaging spectroscopy (Fiorani et al. 2012) and recently sun-induced fluorescence (Rascher et al. 2015). Scaling from the aircraft to the satellite is a question of balancing spatial resolution, repeat time, and coverage. In general, airborne sensors have a higher spatial resolution, can be used upon request, but cover normally only selected flight lines of a few kilometers swath. Satellite sensors for vegetation monitoring generally start at a spatial resolution of a few meters but have the advantage of a regular and large coverage.

3.3 The Need for Environmental Monitoring

The plant phenotype is determined by the interaction of the genotype and the environmental conditions that modulates the expression of the genetic information in time and space (Fiorani and Schurr 2013). As a consequence, plant phenotyping requires an exact monitoring of the environment at the location where the plant is cultivated.

Field phenotyping depends highly on environmental monitoring at the relevant spatial and temporal scales. This may be more complicated than first anticipated. Environmental conditions could be highly variable even on small spatial and temporal scales. One prominent example is light. Light quality and quantity has an inherent diurnal cycle that is deeply imbedded in the regulation of almost all plant functions (Greenham and McClung 2015). Additionally, light constantly changes because of moving clouds, cast shadows, and wind-moved canopies. As a consequence, photosynthesis almost never operates at constant but most of the time under highly fluctuating conditions (Rascher and Nedbal 2006). These fluctuations vary on each leaf and cannot be measured appropriately by light sensors. A second example comes from soil sciences. There is a vast selection of soil moisture sensors, and recent studies demonstrate that soil moisture is very heterogeneously distributed within the soil (Hinsinger et al. 2009). This heterogeneity is important because it determines the actual water availability at fine roots. Both examples demonstrate the importance but also the complexity of environmental monitoring at the "relevant" scale.

In modern field phenotyping, a dense network of environmental sensors is installed. It is attempted to record the most relevant environmental parameters, and currently modern field phenotyping centers have numerous environmental sensors installed. These sensors are often custom made and are positioned as close to the plants as possible. Some prominent examples are the environmental sensors that were developed by the Australian field phenotyping center and Forschungszentrum Jülich (Germany). The major advantage of this concept is that the user can define the type, distribution, and density of the sensors based on the scientific question and thus ensure adequate monitoring of the environmental variability in time and space.

3.4 Conclusions and Outlook: The Future of Field Phenotyping

Currently, several national and international initiatives are on the way to developing infrastructures for field phenotyping. Networks such as the German Plant Phenotyping Network (DPPN),* the French Plant Phenotyping Network (FPPN),† the UK Plant Phenomics Network,‡ the European Plant Phenotyping Network (EPPN),§ or the International Plant Phenotyping Network (IPPN)¶ do not only improve the infrastructure but also develop a new community within plant, vegetation, and agricultural sciences. For instance, the EMPHASIS (European Infrastructure for Multi-Site Plant Phenotyping and Simulation for Food Security in a Changing Climate) has been listed on the ESFRI (European Strategy Forum on Research Infrastructures) roadmap and represents an initiative to synergistically merge the plant phenotyping research infrastructure within Europe. This includes phenotyping facilities under controlled and field conditions as well as modeling and data management. Additionally, EMPHASIS will facilitate a close interaction of different stakeholders from academia and industry including breeders, technology developers, and policy makers. It can be expected that within the next years a network of instrumented field sites will become operational and that field phenotyping data will become widely available from these groups and networks.

* www.dppn.de.
† https://www.phenome-fppn.fr/.
‡ http://www.ukppn.org.uk/.
§ http://www.plant-phenotyping-network.eu/.
¶ http://www.plant-phenotyping.org/.

One of the main challenges will be to merge data from the different initiatives into one knowledge-based environment. However, up to date no standards are available to store, label, and distribute the various data. Success of previous large-scale initiatives such as the human genome project,* the FLUXNET initiative,† or the Long Term Ecological Research Network (LTER)‡ was often linked to a standardized and open data policy.

Phenotyping data are far more complex than what we have experienced before. With field phenotyping, we have to cope with a multitude of data formats and measurement protocols that finally have to be linked to the genetic, environmental, and spatial information resulting in a certain phenotype. But nevertheless linking these fundamentally different data sources is essential to increase our understanding of the gene × environment × management interaction.

Future developments in phenotyping platforms will strongly benefit from sensor arrangements, which allow the fusion of ground and remote sensing data. Particularly the fusion of 3D imaging systems with other sensors like RGB, hyperspectral, or thermal cameras may help to analyze and interpret data with respect to spatial relations between sensor and plant surface. In this context, intensively instrumented field sites, such as the field lab Campus Klein-Altendorf of Bonn University,§ where different sensors and measurement protocols can be tested in an integrated manner, will become highly valuable.

The joint measurements of plant traits will allow the analysis of functional links between phenotypic traits. This becomes especially important if we aim for a better understanding of dynamic plant stress responses. Abiotic as well as biotic stress factors generally result in a complex reaction of the plant that can often not be explained by assessing a few plant properties only. Combined sensors and the possibility to link the data from different experiments give hope that plant phenotyping will also foster our scientific understanding of plant stress physiology. This knowledge in turn is of outermost importance to breed for future plant cultivars that are adapted to the changing and more extreme environmental conditions we are inevitably facing in times of global change.

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* http://www.genome.gov/10001772.
† http://fluxnet.ornl.gov/.
‡ https://www.lternet.edu/.
§ https://www.cka.uni-bonn.de/.

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