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Water use efficiency of arable and grassland crops in legume-based intercropping systems

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Chapter 1: Prologue

Agricultural farming practice in Germany has undergone changes towards less diverse systems with short rotations and few main crops during the last decades (Stein and Steinmann, 2018). Ensuring sustainability with simultaneously increasing yield potential is therefore one of the main challenges for future agriculture (Tilman et al., 2002). Nevertheless, yield progress has declined for several crops in the last years (Fischer and Edmeades, 2010). To further enhance the productivity of agricultural systems, the utilization of genetic resources and biodiversity services has to be improved.

1.1 Legumes in cropping systems

In this context, cropping of legumes can widen existing crop rotations and thus enhance the biodiversity of agricultural landscape. Additionally, fertilizer supported production increases and improvements in independence on fertilizer application is needed (Cazenave, 2018). Legumes with their capability of nitrogen (N) fixation are widely known to be more efficient in the use of soil N and the amount of additional fertilizer can therefore be reduced (Rubiales and Mikic, 2015). As a consequence, seed yield of various crops is increased when the preceding crop was a legume (Tanaka et al., 2007).

Another advantage of legumes is their potential in ecological services (Altieri, 1999; Jensen et al., 2010; Köpke and Nemecek, 2010). The integration of legumes in crop rotations leads to ameliorated soil structure (Rochester et al., 2001), increased microbial activity (Biederbeck et al., 2005) and provision of habitats for beneficial insects (Crist et al., 2006).

Cropping of legumes like pea and faba bean nowadays receives more interest in Europe due to its potential to replace soy bean as protein source for feed and food. To date, Europe is highly dependent on soy bean imports (de Visser et al., 2014; Jensen et al., 2010). Nonetheless, in Germany, legumes such as faba bean (*Vicia faba* L. var. minor) are rarely cultivated as crop rotations are based on cereals. Legumes generate higher complexity for farming management due to soil-borne diseases and possible nitrogen losses via leaching or emissions (Jensen et al., 2010; Reckling et al., 2016).

However, high yielding and stress tolerant legume cultivars are scarce due to low investment in legume breeding (Reckling et al., 2016). There are ongoing studies by several research institutes to translate available genetic resources into novel winter-hardy varieties of faba bean (Link et al., 2010). Those varieties are suitable for a broad range of cropping areas with the ability to make use of a longer vegetation period (Link and Arbaoui, 2005). Frost tolerance plays a major role in breeding of these cultivars, while other factors influencing yield stability receive increasing importance. These are aspects regarding growth conditions, i.e. drought tolerance, as well as pest resistance (Khan et al., 2010; Torres et al., 2006).

1.2 Intercropping systems

At the aim of increasing biodiversity of cropping systems, intercropping of different species provides an opportunity to diversify crop rotations and agricultural landscape. Intercropping systems are defined as growing different crops simultaneously in the same field in variable densities such as substitutive in alternating rows (Andrews and Kassam, 1976). Most often, mixtures of different species result in overyielding of the whole crop stand compared to pure stands such as due to increased interception of light under low interspecific competition (Bilalis et al., 2010; Jolliffe and Wanjau, 1999). Resilience of the system, weed and disease suppression and other factors are also enhanced by the complementarity of legumes and cereals (Carton et al., 2018; Hauggaard-Nielsen et al., 2008, 2001). This complementarity, particularly in differences in plant habitus and flowering characteristics, leads to enhanced biodiversity of associated flora and fauna (Altieri, 1999; Weißhuhn et al., 2017).

Therefore, mixtures of arable crops are to a small share present in existing agricultural systems, especially in organic farms. Where reduced tillage and cropping of legumes is already applied, intercropping systems bear the potential for reduced pesticide inputs (Lemken et al., 2017; Theunissen, 1997). Species mixtures are very common in grassland systems because of yield stability under spatial and seasonal variations (Fowler, 1982). They improve forage quality and higher productivity at late stages of the season (Sleugh et al., 2000). In those systems, nitrogen is used more effectively due to direct transfer from legume to non-legume as well as long term availability by mineralization of crop residues (Olesen et al., 2002; Xiao et al., 2004). That way, nitrogen losses are mitigated and the need for additional fertilizer input is reduced. This nitrogen is transformed into higher protein contents of the harvested products (Bedoussac and Justes, 2010). Also mobilization of soil phosphorus can be promoted in legume-based intercropping systems by associations of mycorrhiza with legume roots or by root-induced pH changes (Betencourt et al., 2012; Ren et al., 2013).

In Germany, selection and breeding for legumes, e.g. faba bean and white clover, is usually performed in pure stands and not in mixtures. Comparing these two crop stands, trait expressions can vary and the best observed performance in pure stands can be different from the best performance in mixtures. This is due to the fact that beneficial effects of intercropping systems interfere with competition for growth resources such as light and water (Gao et al., 2009; Lithourgidis et al., 2011; Malézieux et al., 2009; Mushagalusa et al., 2008). The performance of the system thus depends on environmental conditions and genotypic characteristics (Passioura, 2006). The suitability of a specific genotype is governed by complex interactions. Traits as winter hardiness, competition abilities in comparison to the non-legume, i.e. wheat and ryegrass, and in case of grassland the persistence of the sward therefore need to be tested in mixtures. Consequently, knowledge driven improvement of the performance of such intercropping systems is needed.

1.2.1 Intercropping with faba bean

The cultivation of faba bean is a promising option to replace the high input demanding soybean as locally produced protein source for food and feed (Köpke and Nemecek, 2010). The quality and protein content of cereals can be significantly enhanced when faba bean was intercropped with wheat or durum wheat (Ghanbari-Bonjar and Lee, 2002; Tosti and Guiducci, 2010). Moreover, the yield stability of intercropped faba bean is greater than that of sole cropped faba bean (Hauggaard-Nielsen et al., 2008).

Intercropping of winter types of faba bean and winter wheat have the potential of higher yields due to a longer growth period (López-Bellido et al., 2005) and a better weed suppression than spring types as early canopy closure to compete out emerging weeds (Haymes and Lee, 1999). Yet, the potential of intercropping autumn-sown faba bean and wheat is to date not fully explored as there are only very few winter-hardy varieties on the market, which are able to benefit from rainfall events during winter. On the German market there is only one winter hardy faba bean cultivar available (cv. Hiverna) (Bundessortenamt, 2017). In order to grow faba bean in autumn-sown mixtures with winter wheat, breeding for more adapted cultivars is necessary.

Especially drought adaptation is an issue of recent research as faba bean is a very sensitive crop to water limitations (Belachew et al., 2018; Khan et al., 2010, 2007). Consequently, genotype-specific properties of different faba been genotypes to water deficit can vary in their stress adaptation and drought tolerance (Mwanamwenge et al., 1998). This again would affect the performance of faba bean in the mixture as well as the performance of the intercropping system as a whole.

Structural complexity of faba bean and wheat intercropping systems leads to weed suppression (Ghanbari-Bonjar and Lee, 2003). Nevertheless, weed suppression is less important for intercropping of winter varieties compared to spring varieties, due to its advantage of an early soil cover before weed emergence (Haymes and Lee, 1999). To avoid interspecific competition between faba bean and wheat it is necessary to choose tall growing wheat varieties for better light interception and less shading (Haymes and Lee, 1999). Otherwise, facilitated faba bean growth in intercropping suppresses the accompanying wheat (Lithourgidis and Dordas, 2010). If this is considered, positive effects like an increased proportion of nitrogen derived from N_2 fixation in intercropping of faba bean and wheat compared to pure stands of faba bean are expected (Fan et al., 2006).

1.2.2 Intercropping with white clover and chicory

In grassland, higher plant species richness as present in intercropping systems, leads to an increased biomass production and less investment in root systems, resulting in overyielding of mixtures compared to monocultures (Bessler et al., 2009). Intercropping is therefore a common practice worldwide for pastures, forage and biomass production. Environmental benefits include an increase in biodiversity and a reduced soil erosion by a permanent ground cover (Halty et al., 2017) as well as a decreased risk for annual weeds due to dense vegetation structure and repeated cutting (Weißhuhn et al., 2017). Thereby, high proportions of grasses enhance carbon sequestration by belowground biomass, while high proportions of legumes accumulate more nitrogen (McElroy et al., 2016). This nitrogen can be transferred to non-legumes, especially grasses with fibrous roots (Pirhofer-Walzl et al., 2012).

White clover (*Trifolium repens* L.) as a fast growing species is a strong competitor in grassland systems and therefore avoids weed invasion, leading to increased yield stability (Frankow-Lindberg et al., 2009). Over several years it was shown by Høgh-Jensen and Schjoerring (1997) that after establishment of intercropping, white clover substantially contributes to nitrogen supply for perennial ryegrass in an increasing manner. Additionally, interspecific competition with the accompanying species and seasonal water deficit can lead to declined proportions of white clover in intercropping systems (Hutchinson et al., 1995).

In this context, including species with deep root systems leads to enhanced water availability and in the long term to soil aeration and drainage (Weißhuhn et al., 2017). Deep rooting species such as chicory (*Cichorium intybus* L.) with its tap root system have the capability to take up water from deep soil layers and transfer it to the shallow rooting species (Skinner, 2008). Chicory is used as vegetable but can also be integrated as forage crop into grassland mixtures as chicory has a high yield potential (Hume et al., 1995).

1.3 Drought and water use

The efficient use of water resources is of particular importance under current shifts in precipitation patterns towards extreme weather events (Brouder and Volenec, 2008). This requires sustainable agricultural systems with improved stress tolerance to excess water as well as water scarcity. The latter will be the focus of the present research. In European agricultural systems, the water deficit mostly occurs in a range where plants are still able to maintain leaf water potential and turgor pressure due to stomatal adjustment, root development and reduction in leaf growth (Tardieu, 1996). Thereby transpiration is reduced and water acquisition increased. Yet, these processes lead to reduced yield production (Fita et al., 2015).

For cropping systems with faba bean as well as white clover, water is often the limiting resource (Hutchinson et al., 1995; Jensen et al., 2010). However, legume-based intercropping systems may increase the efficiency of light and water use due to differing stand architecture and alternate rooting (Morris and Garrity, 1993). This way, they contribute to a better realization of yield potential of the crops even under drought conditions.

The yield potential under unfavorable growth conditions can generally be achieved by the adaptation derived from genetic variance transferred into productivity (Boyer 1982). Lobell et al. (2014), however, reported that an increment in yields by breeding for ideal conditions is accompanied by increased sensitivity to drought stress under high vapor pressure deficit. As a consequence, cropping systems nowadays need to gain more stability towards varying climatic conditions.

Breeding strategies for e.g. white clover aim at realizing genetic potential by understanding physiological processes in terms of adaptation to water deficit (Jahufer et al., 2002). Consequently, in order to ensure stable crop production under water limitations, breeding of crops with higher drought tolerance and improved water use efficiency is necessary (Chaerle et al., 2005).

1.3.1 Water use efficiency on the physiological level

Water use efficiency (WUE) can be defined on various scales, depending on the time frame and plant component referred to. It is determined on the leaf level, on the whole plant level and on the crop stand level as general ratio of carbon gain per water loss. On the levels of whole plants as well as crop stands, most definitions of water use efficiency are either related to biomass production and water consumption in various time intervals or to gas exchange of CO_2 and water vapor.

Instantaneous WUE is related to photosynthetic carbon assimilation and water loss via transpiration, which are conversely regulated by stomatal adjustment (Gong et al., 2011). Converted to instantaneous measurements of crop stands in the field, this ratio is expressed as net ecosystem exchange of CO_2 (NEE) per evapotranspiration (ET). A time-integrated approach related to carbon assimilation and transpiration is the stable isotope discrimination against ^{13}C, an indirect parameter to estimate instantaneous WUE (Tambussi et al., 2007).

These measurements of WUE are only reflecting short time intervals and therefore don't include processes of unproductive water loss e.g. during the night. Consequently, WUE evaluated within a short time frame does not necessarily reflect time-integrated WUE, calculated as ratios of biomass per water consumption (Jákli et al., 2016; Tränkner et al., 2016). Here, also parameters such as leaf anatomy, biochemical processes in carbon fixation and night-time respiration contribute to the characteristics of the WUE (Jákli et al., 2017).

1.3.2 Water use efficiency of the crop stand via remote sensing

Information about the production of the cropping system and its water use can be provided by non-invasive techniques such as remote sensing. These techniques are applied in fast screenings of crop stands (Candiago et al., 2015) as it is needed for example as phenotyping tool within the breeding process. The generated information is then used as selection criterion to differentiate genotypes under stress conditions or in various environments (Fiorani and Schurr, 2013).

Other areas of application are the use of near-infrared spectroscopy to predict growth conditions as the carbon and nitrogen content in the soil (Zhang et al., 2017). Furthermore, plant traits for the productivity of the crops in environments with limited resources can be detected (Fiorani and Schurr, 2013). Sensors mounted on tractors then determine the spatial and temporal optimized donation of fertilizer or pesticides to optimize plant growth. The implementation of indices as the Normalized Difference Vegetation Index (NDVI) in this context support the early detection of stress symptoms caused by diseases or other stressors (Behmann et al., 2015).

The NDVI is calculated from light reflectance in the red and the near-infrared range. In the range of the visible light, reflectance is dependent on the absorption of the pigments, foremost chlorophyll, while reflectance in the near-infrared is determined by leaf structure (Carter and Knapp, 2001).

A more distant approach of remote sensing techniques is the use of unmanned aerial vehicles (UAV) in order to estimate yield, species composition and nutrient status of arable and grassland crops (Bendig et al., 2014; Möckel et al., 2014; Möckel et al., 2016). Here, thermal cameras can be used in addition to the abovementioned spectral sensors. These thermal cameras measure the emittance of infrared radiation, detecting the surface temperature and thus the transpiration of crops (Chaerle and Van Der Straeten, 2000).

The canopy surface temperature in the field is highly depending on weather conditions (Mahlein, 2016): wind removes humidity gradient around stomata and increases transpiration, whereas precipitation leads to elevated air humidity and wet surfaces, thus decreasing vapor pressure deficit and reducing transpiration. Elevated air temperature in combination with relative humidity on the other hand increases transpiration demand of the crops.

Consequently, phenotyping of crop stands for productivity as estimated by NDVI and water use as estimated by thermography bear the potential to estimate their water use efficiency and to characterize the system's sustainability. Evaluated under different growth conditions, e.g. several sites and years, these image-based parameters can generate a better understanding of intercropped species and their sustainable production systems.

1.4 Objectives and structure of the thesis

The breeding of new cultivars of crops is traditionally performed in pure stands. This way, the performance of novel genotypes in intercropped stands and responsible traits are not considered. Consequently, growers of arable crops lack information for best management practice of these mixtures. In this framework, the project IMPAC³ (*Novel genotypes for mixed cropping allow for improved land use across arable land, grassland and woodland*) aims at improving knowledge about species mixtures for future breeding of cultivars suitable for intercropping systems.

For the aspect of genotype-specific performance under water limitations, the effects of water partitioning between component crops were evaluated. In this regard, investigations on the efficiency in water use of intercropped vs. pure stands of species mixtures were conducted in arable land and grassland. Due to the complexity of intercropping systems, it is necessary to assess parameters for water use and drought stress tolerance on different scales. Biochemical and physiological mechanisms of plants grown under controlled conditions in the greenhouse provide precise information about responses of the crops to water scarcity. In contrast, data assessment of field experiments considers more complex growth conditions such as weather events, variations in soil structure and occurrence of pests. These findings of both approaches need to be related to each other to obtain detailed understanding of the dynamics in intercropping systems.

Due to its compatible use of resources, intercropping of multiple species is likely to enhance the WUE. This especially accounts for seasons with less water availability. Although there are several studies investigating water use of single cropped faba bean in arable systems (e.g. Abid et al., 2017; French, 2010; Siddiqui et al., 2015), little is known about species interactions in multi species crop stands. This thesis therefore aims at improving the current knowledge on interaction effects of cropping system, species and genotypes with respect to their water use.

In a set of greenhouse experiments, reported in the second chapter of this thesis, contrasting genotypes of winter faba bean were tested under various conditions. The aim was to evaluate their genotype-specific physiological properties and their suitability for intercropping with winter wheat. Growth conditions varied in terms of water availability and fungi inoculation in order to identify stress adaptation with regard to the drought tolerance and the plant microbiome.

In field experiments, various genotypes of winter faba bean as well as white clover in pure stands and intercropped stands with non-legumes (i.e. winter wheat; ryegrass and chicory) were evaluated. In the third chapter, different approaches were used in order to estimate whether the water use and the water use efficiency (WUE) are improved in intercropping systems. These evaluations were additionally compared to non-legumes where nitrogen fertilizer was applied. In this context, it was tested whether intercropping systems have the potential to reduce nitrous oxide emissions in contrast to conventional cropping systems (chapter four). In the same field experiments, drone-based remote sensing approaches of spectral and thermal imaging were applied to observe differences in growth development and water use of the crop stands (chapter five).

Overall, it was to test whether intercropping of the leguminous and non-leguminous species has advantages in comparison to pure stands. Therefore, the cropping systems in general as well as the various genotypes in particular were observed under contrasting growth conditions in order to reveal differences in the performance.

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