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Diagnosing and managing zinc and boron deficiencies in emerging crop production systems of Nepal

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DEDICATION

Dedicated this work to my late mother Chitra Kumari Shrestha. Her love, inspiration and sacrifices have inspired this work.

ABSTRACT

The productivity and sustainability of the prevailing crop production systems are being challenged throughout the Indo-Gangetic Plains. Limiting water resources, depletion of soil fertility, social changes and economic developments drive the current modification of the crop portfolio, reflected in its spatial-temporal patterns and of cultivation practices. In Nepal, this concerns particularly the rice-wheat annual double cropping system, which is the dominant food crop rotation in both the subtropical lowland as well as the temperate Himalayan mid hills of Nepal. As a results of continuing urbanisation and shifting consumer preferences, a drive to replace of wheat with high-value vegetables during the cold dry season is gaining momentum, in the peri-urban fringes., simultaneously, emerging water shortages are preventing permanent soil flooding during the monsoon season, leading to partial substitution of lowland rice by less water-consuming upland crops. Such system shifts and associated changes in soil aeration status are altering the nutrient availability, while increasing the crop demand for the critically limiting micronutrients boron (B) and zinc (Zn).

Therefore, compared the B and Zn levels in the traditional rice- based system (under anaerobic condition), in the water-saving maize-based system (aerobic conditions) with both conventional winter wheat and the emerging vegetables as rotation crops. Under controlled conditions in a dysfunctional greenhouse and under field conditions at two representative production sites and soil types (e.g. Acrisols in Kavre in the mid-hills of Nepal and a Fluvisols in Chitwan in the lowland), determined were(1) differential effects of system shifts on the soil supply and crop demand of B and Zn (diagnosis trials), (2) the effects of applying mineral B and Zn fertilizers on yields and economic returns of wheat vs. cauliflower and tomato (response trials), and (3) longer-term carry-over effects of a one-time application of soil B and Zn on biomass accumulation and nutrient uptake by maize (residual effect trials). Inclusion of an aerobic soil phase (e.g. maize instead of rice) resulted in declining soil C and N contents and consequently negatively affected dry matter accumulation and wheat grain yield. Concurrently, the shift from wheat to cauliflower and tomato increased the demand for B and Zn, and these vegetables showed deficiency symptoms at both sites and in both soil types. Particularly the B concentrations in the biomass of non- amendments crops were always below the critical limits of <10 (wheat), 21 (cauliflower) and 23 mg B kg^{-1} (tomato). In wheat, the application of Zn tended to increase yields under field conditions, while a B application showed no significant effect, irrespective of the site or soil type. On the other hand, biomass accumulation, nutrient uptake, and economic yield of cauliflower and tomato increased with B (and Zn) applications, but response attributes were unaffected by changes in soil aeration status. These responses were generally more pronounced in the lowland than the mid-hill sites, while overall yields of wheat and temperate vegetables were higher in the cool mid-hills than in the subtropical lowland. Despite low application rates of 2.2-4.0 kg ha^{-1} of Zn or B, positive residual effects on subsequent non -fertilized maize were observed with Zn in the Acrisols and with B in both soil types. Soils in larger parts of Nepal are low in available B and Zn. A shift towards aerobic cultivation in the wet season will reduce soil C and N contents and concomitantly the supply of B and Zn. At the same time, the current shift from wheat to vegetables increases the crops' demand for B and Zn. While the application of B and Zn fertilizers can moderately improve the performance of the traditional rice-wheat rotation, with a shift towards vegetable cropping, B and Zn applications become imperative to sustain production. Both the public and the private sectors will increasingly be challenged to develop and make available B- and Zn-containing fertilizer formulations that respond to the changing needs of the emerging production systems. These findings are also pertinent in other environments and for other farming communities in the Indo-Gangetic Plains and the Himalayan foot-hills beyond Nepal.

Diagnose und Management von Zink- und Bormangel in Produktionssystemen Nepals

Die Produktivität und Nachhaltigkeit der vorherrschenden Anbausysteme ist eine Herausforderung für die gesamte Region der Indus-Ganges-Ebene. Begrenzte Wasserressourcen, abnehmende Bodenfruchtbarkeit, sozialer Wandel und wirtschaftliche Faktoren treiben den Wandel von Kulturarten, deren raum-zeitliche Muster, und von Anbaupraktiken in der Region. In Nepal betrifft dieser Wandel in ganz besonderem Maße vorherrschende Reis-Weizen-Rotation, welche das dominante Anbausystem im tropischen Tiefland wie in den temperierten mittleren Höhenlagen des Himalayas darstellt. Die fortschreitende Verstädterung und die sich ändernden Verbraucherpräferenzen führen dazu, dass in der kalten Trockenzeit Weizen durch hochwertiges Gemüse ersetzt wird, während aufkommende Wasserknappheit permanente Bodenüberschwemmungen während der Monsunzeit begrenzt, was zu einer teilweisen Substitution von Nassreis durch weniger Wasser-verbrauchende Trockenlandkulturen führt. Ein solcher Systemwandel reduziert in Verbindung mit Veränderungen des Belüftungszustands des Bodens die Nähstoffnachlieferung, während die neuen Kulturen (Mais in der Regenzeit und Gemüse in der Trockenzeit) den Bedarf an den kritisch limitierenden Mikronährstoffen Bor(B) und Zink (Zn) noch erhöhen wird. Gerade der Mangel an B und Zn wird somit eine der Schlüsselherausforderungen im Zuge des fortschreitenden Umbaus der Produktionssysteme in Nepal.

In Versuchen unter kontrollierten Bedingungen im Gewächshaus wie in Feldversuchen an zwei repräsentativen Standorten (Arcisole im mittleres Hochland und tropisches Fluvisolse im Tiefland) wurden traditionelle Reis-basierte mit wassersparenden Mais-basierten Systemen in Rotation mit Weizen sowie mit Blumenkohl und Tomaten vergleichend bewertet. In einem ersten Schritt wurde der Einfluss der Änderung des Belüftungszustandes des Bodens auf die Nährstoffnachlieferung sowie die Rolle der neuen Rotationskulturen auf den Bedarf an B und Zn ermittelt (Diagnose). Im Folgenden wurde die Pflanzenantwort auf Zugabe von B und Zn Düngern quantifiziert (Düngereffekte). Schließlich wurden Übertragungseffekte einer B und Zn Düngung auf eine ungedüngte Folgekultur von Mais untersucht (Residuale Effekte).

Die Umstellung des Anbausystems von geflutetem Reis auf aeroben Mais während der Regenzeit resultierte in einer signifikanten Minderung des Boden C- und N-Gehaltes und wirkte sich negativ auf die Trockenmassebildung und den Kornertrag von Weizen aus. Die Umstellung von Weizen auf Gemüse erhöhte die den Bedarf an B und Zn, und Gemüse zeigten an allen Standorten typische Symptome für B- und in geringerem Maße auch für Zn-Mangel. Ohne B-Düngung lagen die B-Konzentrationen im Gewebe unter den kritischen Grenzwerten von 10 (Weizen), 21 (Blumenkohl) und 23 mg B kg^{-1} (Tomate). Während der Weizen mit Ertragserhöhung auf eine Zn-Gabe reagierte, konnte unabhängig vom Standort und Bodentyp kein Effekt einer B-Düngung festgestellt werden. Andererseits wurden Biomassebildung, die Stoffaufnahme und der ökonomischer Ertrag von Blumenkohl und von Tomate durch eine Gabe von jeweils 2,0 - 4,4 kg ha^{-1} B und Zn nahezu verdoppelte. Diese Düngeantwort war in der Regel deutlich stärker im Fluvisols im Tiefland als im Acrisols in den mittleren Höhenlagen. Andererseits waren aber die Erträge aller temperierten Kulturpflanzen (Weizen, Blumenkohl und Tomate) in den kühlen Hochlagen deutlich höher als im subtropisch heißen Tiefland. Trotz der relativ geringen Anwendungsrate zeigten sowohl B als auch Zn positive Effekte auf einen nicht gedüngten Mais, wobei solche Residualeffekte von Zn nur im Acrisols und von B auf beiden Bodentypen beobachtet wurden.

Die Böden in weiten Teilen Nepals sind arm an verfügbarem B und Zn. Ein Systemwandel hin zu aerobem Anbau führt zum Abbau von Boden C und N und damit verbunden zu einer verminderten Verfügbarkeit von B und Zn. Gleichzeitig führt ein erhöhter Gemüseanteil in der Fruchtfolge zu einem steigenden B und Zn Bedarf. Während eine B und Zn Düngergabe den

Ertrag von Reis-Weizenrotationen moderat erhöhen kann, ist eine solche Düngemaßnahme im Fall der neuen Mais- und Gemüse-basierten Systeme völlig unumgänglich um eine nachhaltige Erzeugung von Nahrungsmitteln sicherzustellen. Sowohl staatlich Stellen (Agrarforschung und Beratung) als auch der private Sektor (Düngemittelindustrie) stehen unter Zugzwang, B und Zn-haltige Düngeformulierungen zu entwickeln und den Produzenten bereitzustellen, um somit dem fortschreitenden Systemwandel in Nepal und dem sich wandelnden Nährstoffbedarf der neuen Anbausysteme zu begegnen.

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Chapter 1

1 GENERAL INTRODUCTION

1.1 Production systems and system shifts in South Asia

Rice-wheat rotations have for a long time been the most prominent crop production system in the Indo-Gangetic Plains (IGP) and the Himalayan mid-hill regions of South Asia (Figure 1.1). Until recently, they covered 2.2 Mio ha in Pakistan, 9.2 Mio ha in India, 0.4 Mio ha in Bangladesh and 0.6 Mio ha in Nepal (Timsina et al. 2010). This supports the contention that rice and wheat are the main staple foods for South Asia (Ladha et al. 2003) and they are key commodities for regional food security (Chauhan et al. 2012). The two cereal crops are usually cultivated sequentially on the same land and in the same year. Thus, rice is grown during the warm summer monsoon season from June to October followed by winter wheat in the cool and dry winter season from November to April (Becker et al. 2007).

However, in recent years, the productivity and profitability of the rice-wheat cropping sequences have been declining throughout South Asia (Ladha et al. 2007). Several agro-technical and socio-economic drivers were identified for being responsible for recent shifts in the conventional production system and the introduction of new crops and cropping practices. These included (1) progressing urbanization (Rimal et al. 2019) and associated changes in consumption patterns and consumer preferences, (2) a general decline in soil fertility attributes (Harrington 2001), specifically declining soil C and N contents (Ladha et al. 2007), and associated decreases in grain yields (Chatrath et al. 2007), and (3) variable and declining market prices for coarse grains (Jat et al. 2014). These changes have been driving a diversification of the crop portfolio in rice-based systems throughout South Asia (Joshi et al. 2006). In addition, irrigation water is increasingly becoming a limiting factor for sustaining rice-wheat production in the IGP and Himalayan mid-hill regions (Hobbs and Gupta 2003; Tuong and Bouman 2003; Yadvinder-Singh et al. 2014). This continues to be true in particular for areas where surface water is getting scarce for lowland rice production, or where the water table has been declining due to over-extraction for irrigation or domestic and industrial uses (Peng et al. 2009). The high water-demanding lowland rice production is particularly and increasingly challenged by water shortages, and alternative water-saving production systems have been emerging (Aggarwal et al. 2004; Carrijo et al.2017).

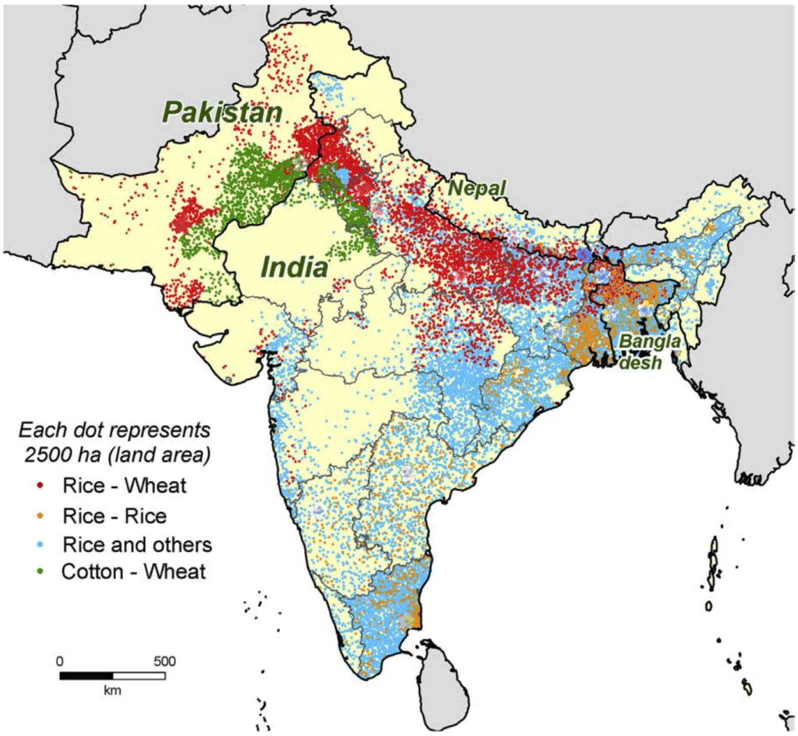

Figure 1.1: Map showing major cereal-based cropping systems in South Asia. Cereal Systems Initiative for South Asia, CSISA-I Project document, International Rice Research Institute, 2009.

Farmers respond to these diverse challenges by gradually modifying their production systems. For instance, in areas where high-value crops can be commercialized (i.e. in peri-urban areas or in those with access to linked markets), farmers increasingly replace the low-value winter wheat by perishable but high-value vegetables such as cauliflower and tomato (Rai et al. 2019). In situations of for instance emerging water shortage, irrigated lowland rice is being replaced by either water-saving rice production strategies (i.e. alternate wetting and drying – AWD, or aerobic rice cultivation) or by a partial replacement of wet season rice by diverse upland crops (e.g. finger millet in higher altitudes, or maize in the mid-hills and lowland).

The changes in both the soil aeration status during the wet season and in the type of cultivated dry and wet season crops entail shifts in the soil nutrient availability and nutrient supply and in the crop nutrient demands leading to changing patterns of soil nutrient extraction and generally in soil fertility in regions concerned. Of prime importance in this context is the inherently low and gradually declining availability of the micronutrients such as zinc (Zn) and boron (B) (Nadeem and Farooq 2019). Moreover, the changes in soil aeration status associated with a shift from irrigated rice in anaerobic soils to mainly aerobic soil conditions in the newly emerging (water-saving) systems is likely to change the plant availability of some nutrients (i.e. P, Fe, Mn and Zn), while at the same time stimulating the microbial mineralization of soil organic carbon, with associated effects on soil water-holding and nutrient retention capacities (Gale and Gilmour 1988).

In Nepal, the agro-ecological conditions in the subtropical lowland region of the Terai and in the subtropical to temperate of the Himalayan foot slopes and lower mid-hills, the climatic conditions and variations create opportunities to grow a wide variety of crops, although maize-based systems in the mid-hills and rice-based systems in the lowland, two cropping system still dominate the agricultural land use (Devkota et al. 2019). The rice-wheat annual double cropping system is yet the most common rotation system throughout the country (Regmi et al. 2009). However, as elsewhere in much of South Asia, recent and recurrent water shortages drive the replacement of lowland rice by water-saving production systems (Shrestha et al. 2013). At the same time, the decreasing productivity and the declining prices for coarse grains (World Bank Group 2016) encouraged farmers of mostly the peri-urban areas to diversify their crop portfolio (Tiwari et al. 2008) and to increasingly cultivate perishable vegetables (Tiwari et al. 2010) to satisfy the growing consumer demand in and around urban centers, particularly for fresh tomato, and cauliflower (MOALD 2019).This shift from a partially subsistence-oriented towards a more market-oriented production benefits smallholder farmers throughout Nepal (Rai et al. 2019), and this shift in systems is gradually replacing the winter wheat in the current crop rotation. However, low to very low contents of particularly the micronutrients B and Zn are prevalent in the mid-hills or the lowland of Nepal (Karki et al. 2005; Andersen 2007) and may be exacerbated with ongoing cropping intensification and diversification strategies and in the absence of appropriate and system-adapted fertilizer recommendations (Sherchan et al. 2005), specifically for micro-nutrients suitable for and targeted to newly emerging cropping systems.

1.2 The role and functions of zinc and boron in crop production

Zn is an essential element for plant growth and reproduction. It plays a key role as a structural component in various enzymes and proteins, and is required thus for various biological pathways (i.e. the carbohydrate metabolism), for photosynthesis, protein and auxin metabolism, for pollen formation and for developing resistance to infection by the certain pathogens (Cakmak 2008a), all of which ultimately affect crop production and yield. Zn also improves the nutritional quality of crop produces, addressing thus the concurrently growing concern for "hidden hunger'', in the country which can be caused by micro-nutrient deficiencies also. Such deficiencies can have devastating effects on human health, especially in children and women (Gregory et al. 2017). The total Zn concentration in dry plant tissues differs obviously by crop type, organ and plant age, but can range from 10 to 300 mg kg^{-1} (Alloway 2008). Zn deficiency is reported as a factor in crop productivity decline in most rice-growing areas globally (Dobermann and Fairhurst 2000; Alloway 2009). It has also been considered as one of the key determinants in reported productivity declines in rice-wheat systems of the IGP region (Nayyar et al. 2001) and is affecting nearly 55% of the rice-growing area of Nepal (Andersen 2007).

Similarly, B is an essential element for plants, playing a major role in the metabolism and transport of carbohydrates, in cell wall synthesis, structure and function, and in the integrity of membranes (Goldbach and Wimmer 2007). It has further been shown to be involved in regulating growth and root elongation via the plant hormone metabolism (e.g. indole acetic acid), being highly concentrated at the growing tips of shoots and roots (Wimmer et al. 2015). Boron is needed in pollen formation as well as in pollination itself, and generally in the reproductive system of plants (Dell et al. 2002). Finally, B is suspected to be involved in the phenol metabolism and DNA synthesis and is several key processes such as protein synthesis, sugar transportation, and respiration (Brown et al. 2002). While B is distributed throughout the earth, its concentrations in soils are generally low and limited to different inorganic forms of borates (Ozturk et al. 2010) – not all of which are plant available. Borate is poorly available in very acid or alkaline soils and its reduced form of boric acid, it can easily be leached, particularly in highly-percolating sand soils (Wild and Mazaheri 1979). In most crop plants, tissue concentrations of B range between 15 and 100 mg B kg^{-1}, which is considered adequate for normal growth. However, at concentrations of >100 mg B kg^{-1}, B can become toxic to plant growth and negatively affect yields (Gupta 2007). While B deficiency is reportedly a major constraint to crop production in at least 80 countries worldwide (Sillanpää 1982), the world's major region of B deficient soils are the Himalayan mid-hills and adjoining lowland areas of the IGP (Rerkasem and Jamjod 2004). This pinpoints the magnitude of the B deficiency challenge for South Asia in general and for Nepal in particular. There, B deficiency reportedly causes sterility in wheat (Srivastava et al. 2000) and generally affects the performance attributes of most non-graminaceous crops.

Since B and Zn are essential for the current crop production (Bell and Dell 2008), and that B and Zn deficiencies already cause significant declines in productivity (Chatterjee et al. 2018), availability of these micronutrients are varies based on depth and elevation and soil pH (Mathew et al. 2016) the application of only small amounts of these micronutrients can reportedly have profound impacts on yields (Mondal et al. 2015). However, no validated fertilizer recommendations are available and such nutrient amendment strategies need to be adjusted to the different agro-ecological zones (Zhang et al. 2020) and the different soil types in Nepal, and they must reflect the ongoing changes in production systems and hence the associated changing nutrient demand. Thus, it is recognized that vegetables require much more B and Zn than for example the conventional rice-wheat rotation (Wimmer et al. 2015).

In principle, only small quantities of both micronutrients (B and Zn) are required to ease the deficiencies that currently limit crop growth and development. However, because of the large differences in Zn needs between crop species and since of the narrow range between B deficiency and toxicity (Singh and Abrol 1985; Goldbach et al. 2000), the correct rates of B and Zn to be applied because of avoiding both toxic or deficiency effects, are highly site, crop- and system-specific (Singh and Abrol 1985; Gupta et al. 1985). Another consideration related to the low amounts of B and Zn needed concern the application frequency. Applying B and Zn once a year will probably suffice for the current and subsequent crop in the rotation, and even longer-term residual effects of one-time applications of B and Zn have been reported (Yang et al. 2000; Amanullah and Inamullah 2016). This is of particular importance in environments and in production systems where appropriate B and Zn fertilizer are not easily accessed and where precarious economic situations of smallholder or low-input farmers limit the purchase and regular application of such micro-nutrient formulations. This will be the case for most of Nepal and its majority of small-scale producers.

1.3 Problem statement

With diverse production environments (i.e. mid-hills and lowland area) and predominant soil types (i.e. Acrisols and Fluvisols) and with wide-spread deficiencies of B and Zn in most soils of Nepal, the need for a site-/soil-specific assessment B and Zn soil supply and crop demand, and resulting fertilizer recommendations becomes apparent. The urgency for such knowledge is increased by recently on-going shifts in the dominant rice-wheat production system. Thus, the inclusion of vegetables in existing rotations or the replacement of winter wheat by perishable high-value commodities may differentially change the demand for key limiting nutrients (Khai et al. 2007). The additional change in cultivation practices and the partial substitution of irrigated lowland rice by upland crops entails changes in soil aeration status that again are likely to differentially affect the availability of B and Zn in the soil. Thus, the demand for Zn and its removal by lowland rice is usually high. On the other hand, the rice demand for B may be low, but B losses by leaching are likely to occur and being enhanced particularly in coarse-textured soils (Broadley et al. 2012) upland crops or aerobic production methods will thus entail a reduced Zn demand but also a reduced soil Zn availability in aerobic soils. On the other hand, water-saving crop production may reduce B leaching losses while upland crops and particularly vegetables will substantially increase crop B uptake from the soil.

Little is known regarding the nutrient status of different soil types and critical threshold values of different crops (B and Zn diagnosis). No recommendations exist for B and Zn application, especially those differentiating crops, rotations, and sites. There is a need to consider both the supply and the demand of the limiting micronutrients with the production site, to the soil aeration status, and the main dry season crops (B and Zn response). Finally, B, and to a lesser extent Zn, formulations are not available on local markets, they may not be available on time, and they are often not affordable by smallholder farmers. Thus, single small applications with longer-term effects for subsequent crops are desirable, provided that they entail financial gains and economic benefits (B and Zn residual effects). In summary, there is an urgent and growing needs for the site- and system-specific nutrient diagnosis and management strategies and eventually for fertilizer recommendations to smallholder producers in Nepal.

1.4 Hypothesis and objectives

Research hypothesize that the widely-reported B and Zn deficiencies in Nepal differ in their extent by soil type and region and that the on-going shift towards the new emerging production systems with changing soil aeration status and new crop types in the rotation will entail different B and Zn supply from the soil, B and Zn demand by the crop and different residual effects of one-time fertilizer applications. Such knowledge is essential and urgently required to foster the emerging production systems for the challenges ahead, and for supporting smallholder farmers with scientific knowledge-based recommendations that allow them a sustainable transition towards diversified and intensified food crop production in Nepal. The targeted application of B and Zn fertilizers on selected dry season crops in the dominant rotation rice-wheat will increase yield and consequently financial gains to farmers in different growing environments of Nepal. To test this hypothesis and to provide the scientific basis for B and Zn fertilizer recommendations, the following objectives were addressed (a) in the conventional rice-wheat and the emerging maize and/or vegetable-based systems, and (b) in soils of both the mid-hills and the lowland region of Nepal:

1. Assessing the soil status of available B and Zn and the critical tissue thresholds and responses of major crops to B and Zn in the greenhouse and at two field sites (B and Zn diagnosis).
2. Determine the response of wheat and vegetables (cauliflower and tomato) to applied B and Zn in the greenhouse and at two field sites (B and Zn response).
3. Analyze the possible carry-over effects of previously applied B and Zn on maize grown in potted soil in the greenhouse (B and Zn residual effects).

1.5 Structure of the thesis

This research will answer the questions to how micro-nutrients supply and demand are affected by climate (mid-hills vs lowland), soil type (Acrisols vs Fluvisols), and individual crop demands by wheat, cauliflower and tomato in Nepal and how these can be synchronized in a technically feasible and economic viable way.

According to the hypothesis and the objectives defined, this thesis is structured into the following sections.

This **General Introduction** Chapter provides the background to rice-wheat systems in South Asia, to the emerging system shifts that have been recently observed, and the roles and functions of the key limiting micronutrient elements B and Zn. The Chapter ends by formulating the hypothesis and stating the objectives that will be addressed by empirical research activities in both the greenhouse and under field conditions at two representative sites of Nepal.

In the **Methodology** Chapter details of the methodologies employed in the different steps of this study and gives an introduction of the study site, its physiographic condition.

The first result (**Diagnosis**) presents the findings of the diagnosis experiments, entitled "Diagnosis of Boron and Zinc availability in emerging vegetable-based crop rotation in Nepal" and published in 2020 as: Shrestha S, Becker M, Lamers JPA, Wimmer MA (2020a) Diagnosis of Zinc and Boron availability in emerging vegetable-based crop rotations in Nepal. Journal of Plant Nutrition and Soil Science 183:429–438. https://doi.org/10.1002/jpln.202000020

The second result Chapter (**Response**) presents the study on B and Zn applications in both the field and the greenhouse. The study has also been published in 2020 as: Shrestha S, Becker M, Lamers JPA, Wimmer MA (2020b) Boron and zinc fertilizer applications are essential in emerging vegetable-based crop rotations in Nepal. Journal of Plant Nutrition and Soil Science 183:439–454. https://doi.org/10.1002/jpln.202000151

The third result chapter addresses the (**Residual**) effects of a one-time B and Zn application on a crop of maize, grown in potted soils in the greenhouse. The Chapter has been submitted for publication in June 2020 to the Journal of Plant Nutrition and Soil Science as: Shrestha S, Becker M, Lamers JPA, Wimmer MA (2020) Residual effects of B and Zn fertilizers applied to dry season crops on the performance of the follow-up crop of maize in Nepal. Journal of Plant Nutrition and Soil Science. (Paper accepted, jpln.202000289).

The last Chapter in the **General Discussion** that summarizes the key findings and discusses them in relation to the initially set hypothesis. The thesis completes with addressing the salient and overwhelming findings and the main conclusions and implications of the findings and outlook for further research and practical implementation. In the appendices, additional materials are included as separate items. As well as pictures showing and illustrating key findings of the pot and field trials.

LIST OF PUBLICATIONS INCLUDED IN THIS THESIS

Shrestha S, Becker M, Lamers JPA, Wimmer MA (2020a) Diagnosis of Zinc and Boron availability in emerging vegetable-based crop rotations in Nepal. Journal of Plant Nutrition and Soil Science 183:429–438. https://doi.org/10.1002/jpln.202000020

Shrestha S, Becker M, Lamers JPA, Wimmer MA (2020b) Boron and zinc fertilizer applications are essential in emerging vegetable-based crop rotations in Nepal. Journal of Plant Nutrition and Soil Science 183:439–454.
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Shrestha S, Becker M, Lamers JPA, Wimmer MA (2020c) Residual effects of B and Zn fertilizers applied to dry season crops on the performance of the follow-up crop of maize in Nepal. Journal of Plant Nutrition and Soil Science. (Paper accepted, jpln.202000289)

Shrestha S, Becker M, Lamers JPA (2019a) Effects of emerging crop rotations and changing soil aeration status on B and Zn availability and vegetable responses in Nepal. In: Tielkes E (ed) Filling gaps and removing traps for sustainable resources development. Tropentag, Kassel, Germany

Shrestha S, Becker M, Lamers JPA (2019b) Emerging cropping systems affect soil boron availability and crop response in Nepal. In: Research and Innovation for prosperity. Nepal Academy of Science and Technology (NAST), Kathmandu, Nepal

Shrestha S, Becker M, Lamers JPA, Wimmer MA (2020d) Emerging crop rotations affect soil B availability and increase crop response to applied B in Nepal. In: International symposium on boron in agriculture, Antalya, Turkey (extended abstract submitted)

Chapter 2

2 GENERAL MATERIAL AND METHODS

2.1 Study region

Agriculture is the main source of Nepal's economy, contributing >33% of the Gross Domestic Product (GDP), and 66% of the population depends on agriculture for their livelihood (MOF 2020). Most farmers have small landholdings with a subsistence-production orientation, and farming systems are mostly cereal-based, with rice, wheat, maize and finger millet being the dominant crops; however, this crop dominance varies with elevation and agro-climatic zone. Irrigated rice and wheat are grown in an annual rotation, and together with rainfed maize are cultivated in the lower mid-hills and the subtropical lowland of the Terai (the generally flat area bordering, the Indian border) finger millet and barley, but also rainfed wheat are grown on sloping uplands up to 4,500 m above the sea level. The mid-hills and the lowland (Terai) regions are the main production regions of the country with a clear dominance of the annual rice-wheat double cropping rotational system (Khatri-Chhetri and Aggarwal 2017).

However, in recent years, Nepalese farmers have been expanding their crop portfolio, partially to respond to changing market demands and price, but also as a risk alleviation strategy. As a result, vegetable farming is gaining importance in the vicinity of the larger urban centres or in areas with good transport infrastructure that reduces market transaction costs. The area under vegetables has increased by 41% in the past 10 years making vegetable cultivation the fastest-growing agricultural sector in Nepal (CBS 2013). Cauliflower and tomato are first and third in terms of area and production (MOALD 2019).

2.1.1 Study sites

The experimental sites were selected in areas under the jurisdiction of the Nepal Agricultural Research Council (NARC), which is the government-managed national agricultural research organization in Nepal, responsible for conducting agricultural research and coordinating extension services in the country. Pot experiments were conducted in a National Soil Science Research Centre NARC-owned greenhouse in

Kathmandu, while field trials were established at two NARC research station sites; these evaluation sites had contrasting edaphic and climatic conditions, one representing the mid-hills and the other the lowland area of Nepal. Both sites have a history of long-term land uses with rice-wheat and maize-based rotations (see Appendix A2). The experimental plots in the mid-hills site were established on farmer's fields of the village of Kuntabesi, within Mandandeupur Municipality of Kavrepalanchowk District. This village is part of an outreach site of NARC. It is located approximately 55 km from Kathmandu at 27°42' N latitude and 85°36'E longitude, and at an altitude of 800 m. The site in the lowland was located on the experimental station of NARC's National Maize Research Program of at Rampur, Chitwan District, at 27°65'N latitude and 84°34'E longitude, and at an altitude of 228 m (see Appendix A5). The geographical location of the study sites is shown in Figure 2.1.

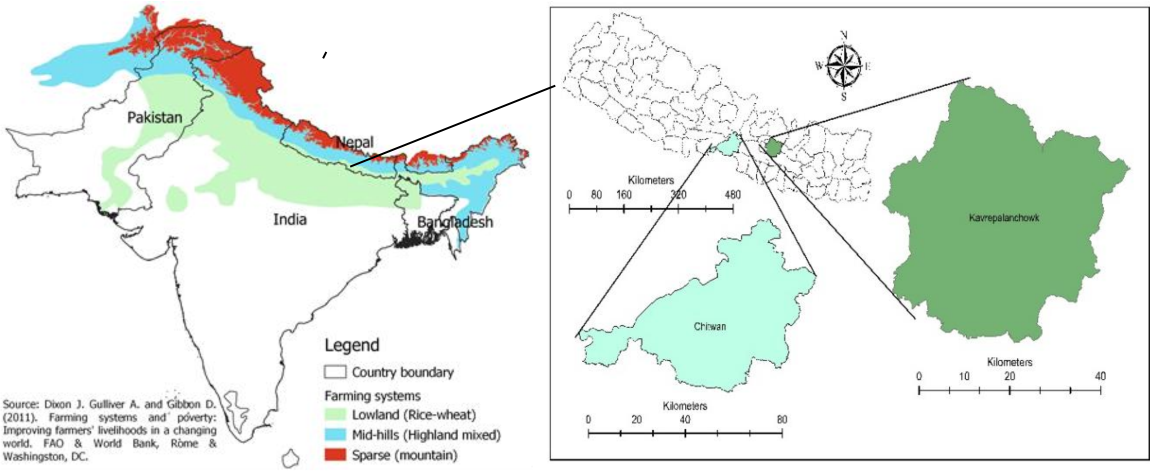

Figure 2.1 Map showing the country Nepal and the experimental field sites, representing the mid-hills and the lowland region of Nepal.

At the mid-hills site in Kavre rice, maize, wheat, finger millet and potatoes are the dominant crops, while cropping at the lowland sites in Rampur is dominated by rice, wheat and maize (Table 2.1). In recent years, due to improved market access and changing consumer demands, vegetable farming has become increasingly popular at both sites (CBS 2018).

Table 2.1: Socioeconomic and biophysical attributes of the study sites in Nepal.

Parameters	Mid-hills (Kavre)	Lowland (Chitwan)
Population of study sites	381,937	579,984
Average area of landholding (ha)	0,5	0,7
Cropping intensity	1,8	1,8
Area under paddy production (ha)	11,310	31,942
Area under wheat production (ha)	8,925	6,020
Area under cauliflower production (ha)	1,236	433
Area under tomato production (ha)	2,623	632

(CBS 2018)

2.2 Climate

While Nepal covers a wide variation of landscapes, altitudes, topographic features, and climatic conditions, only three agro-ecological zones are recognized to be of importance and contributing to national food security i.e. the Mountains, the Mid-hills and the Lowland (Terai) (Figure 2.2); each has their specific altitude and temperature environments and specific soil formations. Throughout the country, December and January are the coldest months, while June and July are the hottest months. Most rainfall occurs during the monsoon season, usually between June and October. Both, the annual rainfall, and temperature differences of the different regions strongly affect crop suitability and production.

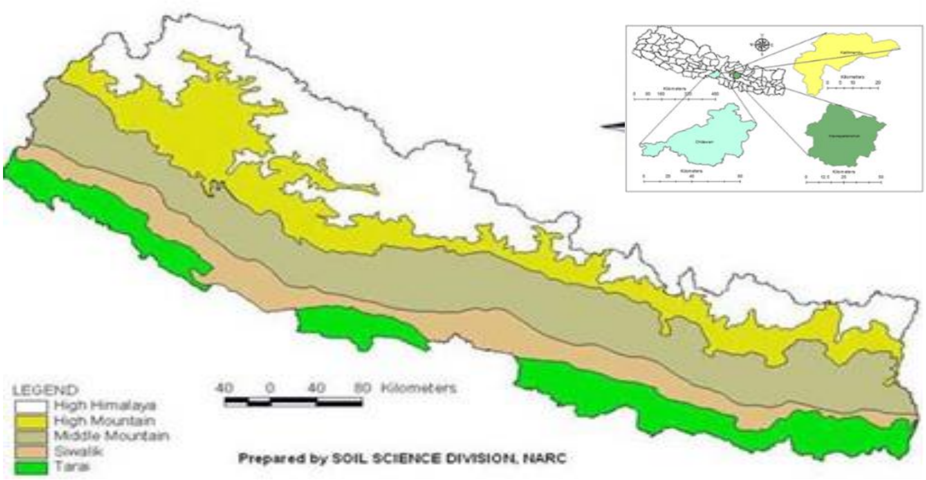

Figure 2.2: Map showing the physiographic regions of Nepal.

In the rain-sheltered greenhouse in Kathmandu, mean temperatures during the study period ranged from 20 to 25°C with a relative humidity of approximately 70%. The mid-hill experimental sites were characterized by a warm-temperate climate with mean maximum temperatures ranging between 28 and 30°C during the summer, approximately 10°C (mean minimum) in the winter season, and an annual rainfall of 879 mm. The lowland site has a subtropical climate with maximum summer temperatures ranging from 30 to 35°C, and mean minimum winter temperatures of 9°C, and annual precipitation of 1,760 mm. Maximum temperatures at both field sites usually occurred in the pre-monsoon period in March or April while rainfall peaked during the monsoon season between June and September (Figure 2.3).

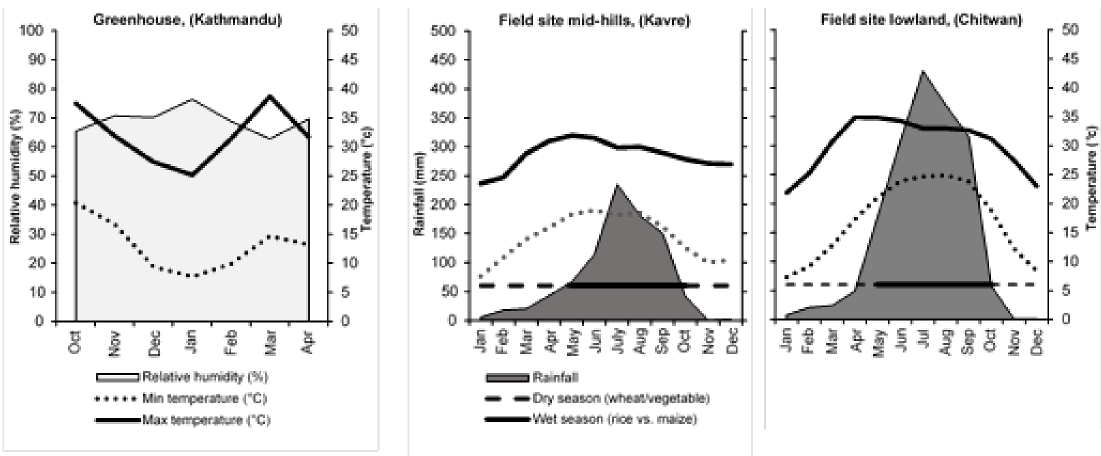

Figure 2.3: Climatic conditions (e.g. long-term monthly averages of minimum and maximum temperatures and rainfall at the field sites between 2007-2018, and monthly relative humidity and mean minimum and maximum temperatures in the greenhouse recorded in 2017); crop growing seasons are shown for the greenhouse (Kathmandu, left) and at the field experiment sites in the mid-hills (Kavre, middle), and the lowland (Rampur, right) in Nepal.

2.3 Soil

Soil formation in the study region are governed by a combination of parent rock, weathering processes and elevation (Bäumler and Zech 1994). Pedologically, most of the soils are derived from phyllite, schist, or quartzite, leading to moderately low inherent soil fertility. The areas dominated by sandstone and granite have developed poor sandy soils, which are not suited for rice cultivation or irrigated agriculture.

According to the World Reference Base (FAO 2014), the soils at the experimental sites are classified as Acrisols in the mid-hills and as Fluvisols in the lowland. The Acrisols with sandy clay loam textures dominate the mid-hill areas of central and eastern Nepal, while Fluvisols, characterized by sandy loam textures, prevail in much of the lowland (Terai) (Andersen 2007). The high intensity of the monsoon rains causes leaching losses of all the major cations, which increases soil acidification-leaching of boric acid also occurs (Ghimire and Bista 2016). More than 50% of the soils are acidic and highly weathered with low availabilities of macronutrients and low soil stocks of the plant available micronutrients (Alloway 2009). As a consequence, more than 50% of the agriculturally-used land area of Nepal is covered by soils that are currently characterized by B and Zn-deficiencies (Andersen 2007). Crop cultivation without the addition of externally applied nutrients provides low yields.

To characterize the soil at the experimental sites, the pH values were determined in water (1:2.5 volumetric ratio); total C and N contents of the soils were determined using a EURO EA Elemental Analyzer, Series 3000 (Euro Vector); available Zn was determined through DTPA extraction (Lindsay and Norvell 1978); soil B was determined from $CaCl_2$ (0.01 *M*) extracts using a miniaturized curcumin method. The soil at the mid-hills was an acid sandy clay loam (pH 4.3), while that at the lowland was a slightly acid sandy loam (pH 5.7). The soils' total nitrogen contents were comparatively higher in the rice-based than in the maize-based system (Table 2.2).

Due to their physical proximity the soils of the maize- and the rice-based system sites were comparable, and existing differences in soil characteristics were assumed to have been caused solely by changes in land use for either irrigated lowland rice or upland maize during the past 3 to 5 years. Such crop cultivation effects resulted in differences in the soils' C and N contents as well as in available Zn and extractable B.

Table 2.2: Physico-chemical characteristics of the topsoil layer (0-20 cm) from the mid-hills and the lowland experimental sites in Nepal (samples were collected on May in 2017 before treatment applications).

Parameters	**Mid-hills**		**Lowland**	
Soil classification	Acrisols		Fluvisols	
Texture	Sandy clay loam		Sandy loam	
Sand (%)	45		65	
Clay (%)	33		17	
Silt (%)	22		18	
pH (H_2O)	4.3		5.7	
Preceding wet season crop	**Rice**	**Maize**	**Rice**	**Maize**
Total C (%)	1.7	1.3	1.8	1.3
Total N (%)	0.16	0.12	0.16	0.10
Available Zn (mg kg^{-1})	2.0	3.3	1.9	2.3
Water-extr. B (mg kg^{-1})	0.20	0.30	0.14	0.28
Exch. K (mg kg^{-1} soil)	116	134	78	133
Available P (mg Kg^{-1})	31	63	19	48
Available K (mg kg^{-1})	46	132	50	62

2.4 Land use

Both field experimental sites had been previously cultivated for more than one decade under rice-wheat rotation. In the mid-hills, two adjacent field terraces were selected on the same mountain slope, and these were formed from the same parent material and with similar environmental/climate conditions, and comparable cropping histories; however, the wetter terrace was continuously used for rice-wheat rotations, while the drier terrace had been converted into a maize-wheat rotation in 2014. In the lowland area, one of the two adjacent field sites remained under rice-wheat rotation, while the other had been integrated into the experimental station of the National Maize Research Program (NMRP), and was used in the past decade for maize cultivation during the wet season.

2.5 Study design

The crop responses to fertilizers were assessed by comparing Zn and B application strategies in combination with the recommended sole use of N P and K fertilizers. Pot experiments were conducted to analyse the soil and crop responses to the applied treatments under climate-controlled conditions; thus reduced the prevailing spatial variability in soil attributes, and provided more homogenous conditions for the nutrient supply of the soil (supply site) and the nutrient uptake by the crop (demand site). Based on the identified supply and demand of nutrients, the subsequent on-station field experiments were implemented at the two representative study sites for further validating the soil supply effects and for assessing soil fertility management practices. The residual effect experiment was conducted only as a pot trial in the greenhouse of the Nepal Agricultural Research Council (NARC) in Kathmandu. The overall methodological approaches applied in the research are presented in Table 2.3.

2.6 Origin and attributes of plant materials

Plant materials for the wet season crops of rice and maize and for the dry season crop of wheat were obtained from NARC, while the dry season vegetable i.e. cauliflower and tomato seeds, were obtained from a local agro-vet store in Kathmandu.

2.6.1 Wet season crops

The widely used indica-type rice (*Oryza sativa* L.) varieties “Makwanpur-1” (mid-hills) and “Sambha Masuli Sub-1” (lowland) were recommended from the Agronomy Division of NARC in Kathmandu. Both varieties were released in 2006 and are high-yielding semi-dwarfs with growth durations of 137-150 days. Seeds of the improved maize (*Zea mays* L) variety “Arun-4” were obtained from the National Maize Research Program in Rampur, Chitwan. This variety was released in 2015 and has yellow grain color and growth duration of approximately 115 days.

Table 2.3: Overview of major methodological approaches

Experiment	Objectives	Treatment factors	Hypothesis	Details in section	Location	Picture for visualization
Pot experiment	Assessing the soil status of available B and Zn and the critical tissue thresholds and responses of crops **(B and Zn diagnosis)**	❖ Agro-ecological zone, ❖ Prior cropping system, ❖ Wet season crop (rice, maize) ❖ Dry season crop (wheat, cauliflower, and tomato), ❖ Fertilization (B and Zn)	Ongoing system shifts in Nepal, within the dominating rice-wheat crop rotation system, will substantially increase demand of the widely deficient B and Zn minerals.	Chapter 3 and 4	Greenhouse, National Soil Science Research Centre, NARC Kathmandu	 Tomato growing in pot experiment, Kathmandu
Field experiments	Determine the response of wheat, cauliflower, and tomato to applied B and Zn fertilizers **(validation of results)** **(B and Zn response**)	❖ Agro-ecological zone, ❖ Prior cropping system, ❖ Wet season crop (rice, maize) ❖ Dry season crop (wheat, cauliflower, and tomato), ❖ Fertilization (B and Zn)	B and Zn fertilizers improve yields of dry season crops such as cauliflower, tomato, and wheat.	Chapter 3 and 4	Mid-hills (Kavre), lowland (Rampur)	 Cauliflower growing in the field experiment, mid-hills
Residual effect trial	Analyzing the possible carry-over effects of previously applied B and Zn **(B and Zn residual effects)**	❖ Agro-ecological zone, ❖ Prior cropping system, ❖ Prior dry season crop ❖ Wet season crop (maize), ❖ residual fertilizers	The carry-over effect of the residual fertilizers adds significantly to the overall effect of B and Zn fertilization	Chapter 5	Dysfunctional greenhouse, National Soil Science Research Centre, NARC, Kathmandu	 Maize growing in pot experiment, Kathmandu

2.6.2 Dry season crops

The wheat (*Triticum aestivum* L.) variety "Munal" was obtained from the Agricultural Botany Division of NARC. It originated from CIMMYT and was released in Nepal in 2018. It is a short-statured, high-yielding rainfed variety with a growth duration of approximately 164 days and reported is resistant to rust (Ug99) and suitable to both mid-hills and lowland region of Nepal (Joshi et al. 2017).

The cauliflower (*Brassica oleracea*) genotype "Rami" is an F1 hybrid, popular among farmers and consumers and recommended for the months November-February in Nepal with a growth duration of 80-95 days and is recognized for its market quality attributes of a large and white coloured curd. The tomato (*Lycopersicon esculentum*) genotype "Dalila" is an F1 hybrid was registered in 2010 and is becoming increasingly popular in Nepal due to low incidences of viral and fungal diseases and low damage rates from fruit borers. It manifests as an erect bushy growth habit and round to slightly egg-shaped fruits. "Dalila" is recommended for field establishment in October to supply the market in March / April both in lowland and mid-hills. Establishment dates differed between sites and growth durations of the pre-crops. Thus, at the mid-hill sites, post-rice crops were established on 10 December, while post-maize crops were seeded on 15 November. In the lowland, post-rice crops were established on 5 January, while post-maize crops were seeded on 20 November (Figure 2.4). After harvesting the dry season crops in the pots, non-fertilized maize (*Zea mays* L.) namely the improved genotype Manakamana-4 was seeded on 15th of June. The variety was released in 2008 and popular among farmer due to high yielding and resistance to lodging**.**

2.7 Field measurement and sample processing

Crop yields were determined by harvesting each plant in the pot experiment. In the field, while harvesting the net plots in each treatment avoid the border plants. The fresh weight of all harvested fractions was weighed with a portable scale in the field, topsoil (0-20 cm) samples at each field sites were collected at the onset of the wet season in 2017. Samples were air-dried for 3-4 days and consequently sieved (2 mm mesh size).

In pot experiment, the three plants were grown in each pot and one plant was chosen during early reproductive stage (at 60 DAS), whole plants were cut at the base. Three plants of cauliflower and tomato and 4-5 plants of wheat were uprooted excluding border plants from each plot for plant analysis in the field experiment. The fresh aboveground biomass was chopped, mixed, and weighed with a portable scale and sampled/sub-sampled. Oven-dried above-ground biomass samples (60˚C for 2 days) of plants collected from both the greenhouse and the field trials at the early reproductive growth stage (60-90 days after seedling) were ground to <0.5 mm in a sample mill (FT102 Micro Soil Disintegrator).

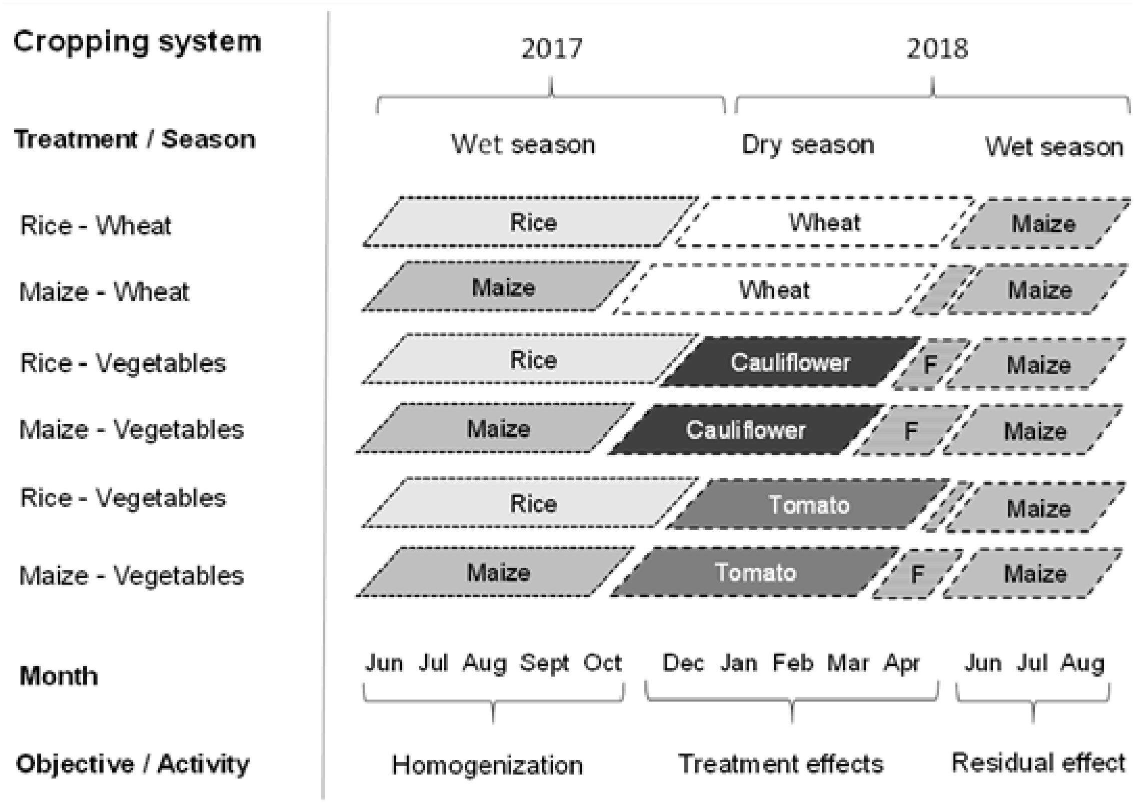

Figure 2.4: Cropping systems (crop types and their sequential arrangement and timing) in both field sites and the greenhouse in Nepal (F = fallow).

(**Note**: Treatments details are described in Table 2.4)

2.8 Laboratory analysis using A miniaturized curcumin method

Soil B was determined from $CaCl_2$ (0.01 M) extracts. Air-dried 25 g soil was taken and added 50 ml $CaCl_2$ (0.01 M) and shaking for one hour (Jeffrey and McCallum 1988) and 100 µl of the filtrate solution was used for determining B content by using a miniaturized curcumin method (Wimmer and Goldbach 1999).

Plant material (500 mg) was pressure–digested in PTFE digestion bombs after addition of 4 mL of HNO_3 (conc.) for seven hours at 180°C. After cooling, digests were transferred into plastic storage bottles. The Zn content of the ash solutions was determined by Atomic Absorption Spectroscopy (AAS). The B content was determined spectrophotometrically by a miniaturized curcumin method (Wimmer and Goldbach 1999). 100 µl of the digestate was acidified with 100 µl (0.1 N HCl) and 50 µl extraction solution (10% 2-ethyl-1, 3-hexanediol in chloroform) added. After complete separation of the two phases, 20 µl were pipetted from the lower, apolar phase and acidified with 200 µl (ml) of 1:1 mixture of H_2SO_4 (conc.) and acetic acid (conc.). After adding 250 µl of the 0.2% curcumin solution and stopping the reaction by adding the 500 µl of Milipore water (B free water), the color formation was detected using a microplate reader (Powerwave XS2, Biotek Instruments, GmbH Germany) at 550 nm. To avoid B contamination from glassware, only plastic laboratory ware was used.

2.9 Nutrient uptake calculation

Plant growth and development largely depend on the concentration of mineral nutrients available in the soil. Plants often not able to be obtaining an adequate supply of these nutrients to meet the demands of the crop. For the calculation of the Zn and B uptake, the Zn and B content (mg kg^{-1}) in dry biomass was multiplied with the respective dry weight (gm^{-2}) of the plant (equation 2.1).

Zn and B uptake (mg m^{-2}) = Dry matter (kg m^{-2}) X Zn or B content (mg kg^{-1})………..(2.1)

2.9.1 Fertilizer application

In the absence of appropriate system-and crop-specific micro-nutrient recommendations in Nepal, the amounts applied in the study were derived from published literature. In general, 1-3 kg B ha^{-1} is considered sufficient for obtaining high yields of annual crops (Martens and Westermann 1991; Shorrocks 1997). However, cauliflower being a B-sensitive crop requires larger quantities than other annual crops. Thus 4.4 kg B ha^{-1} are recommended to be applied in sandy loam soils (Martens and Westermann 1991; Shorrocks 1997). Tomato requires reportedly 2.5 kg B ha^{-1} for optimum production under field condition (Dursun et al. 2010). Similarly, the addition of 20 kg Zn ha^{-1} (Zn sulphate) reportedly increases the yield of tomato (Singh et al. 2016). In wheat, an application of 20 kg Zn ha^{-1} is considered sufficient as under low soil Zn condition (Cakmak et al. 1996; Bharti et al. 2013). Hence, the amount of Zn application for wheat, cauliflower and tomato were adjusted to 15, 20, 20 kg Zn ha^{-1}, while B was applied at 2.0, 4.4, and 2.2 kg B ha^{-1}, for wheat cauliflower and tomato, respectively. The experimental design as well as crop and soil management strategies are summarized in Table 2.4.

Table 2.4: Experimental design, treatments, and management practise in the pot, field, and residual experiments during dry season 2017 and 2018.

Designations	**Pot experiment**	**Field experiment**	**Residual effect trial**
Treatments	NPK, NPK+Zn, NPK+ B, NPK+Zn+B	NPK, NPK+Zn, NPK+ B, NPK+Zn+B	Same layout as pot experiment
Experiment design	Completely Randomized Design (CRD)	Split plot	Completely Randomized Design
Replication	4	4	4
Test crop	Wheat, cauliflower and tomato	Wheat, cauliflower and tomato	Maize
Plot size/Pot size	10-L plastic pots	6m*3m in lowland and 3m*3.5m (Rice-based) and 2.7m*4.2m (maize-based) in mid-hills.	10-L plastic pots
Soil type	Acrisols in mid-hills, Fluvisols in lowland	Acrisols in mid-hills, Fluvisols in lowland	Acrisols in mid-hills, Fluvisols in lowland
Planting date	26th of September- 2nd week of October	10-12th of November and 21st March	15th of May
Fertilizer rate	NPK fertilizes as national recommended. Zinc: 3.3 kg ha^{-1} for wheat, 4.4 kg ha^{-1} for tomato and cauliflower; Boron: 2.0 kg ha^{-1} in wheat and 4.4 kg ha^{-1} for cauliflower and 2.2 kg ha^{-1} for tomato	NPK fertilizer applied as national recommended. Same dose and source of B and Zn applied as the pot experiment	No fertilizers
Fertilizer application	Per pot	Pit application during land preparation (cauliflower and tomato); broadcasting before sowing (wheat)	No fertilizers
Irrigation	Weighting each pot every 2-3 days to determine the water to be applied as the equivalent of the weight loss	FAO CROPWAT 8.0	Weighting each pot every 2-3 days to determine the water to be applied based on weight loss
Topdressing	Two split doses of N fertilizer (urea)at 20 DAS and 40 DAS	Two split doses of N fertilizer at 20 DAT and 40 DAT	No fertilizers
Plant sampling	Early reproductive stages (60-65DAS)	Early reproductive stages (60-65DAT)	Vegetative stage (40 DAS)
Hoeing and staking	Hoeing at 30 DAS, tomato staking at 35 DAS	Hoeing at 30 DAT, tomato staking at 35 DAS	Not applicable
Harvesting	Cauliflower 75 DAS, tomato 150-170 DAS, wheat 150 DAS	Cauliflower 75 DAT, tomato 150-170 DAT, wheat 150 DAS	42 DAS

Chapter 3

Based on the journal article:

Shrestha S, Becker M, Lamers JPA, Wimmer MA (2020a) Diagnosis of Zinc and Boron availability in emerging vegetable-based crop rotations in Nepal. Journal of Plant Nutrition and Soil Science 183:429–438. https://doi.org/10.1002/jpln.202000020

3 DIAGNOSIS OF ZINC AND BORON AVAILABILITY IN EMERGING VEGETABLE-BASED CROP ROTATIONS IN NEPAL

Abstract

Nepal's traditional rice-wheat rotation systems are subject to continuing changes. Changing consumer demand currently drives a replacement of wheat by high-value vegetables during the dry season, while emerging water shortages lead to a substitution of rice by maize in the wet season. Hence associated changes in soil aeration status and shifting conditions of soil nutrient supply to match crop nutrient demand are expected to increase the requirements for the principle limiting micro-nutrients such as B and Zn. Our aim was to investigate the changes in B and Zn availability as well as crop yields and nutrient uptake after system shifts from rice to maize and from wheat to vegetables. Therefore, analyzed the B and Zn availability in rice- and maize-based systems as well as crop yields and the nutrient uptake by wheat, cauliflower, and tomato during the dry season in Nepal. Plants were grown at two field sites (mid-hills vs. lowland) and under greenhouse conditions using soils from the field sites. A change from irrigated rice to maize reduced soil C and N contents with resulting decreases in dry season crop yields. Low soil Zn after rice cultivation led to a shortage in Zn uptake by vegetables in both greenhouse and field experiments. The shift from wheat to vegetables increased the demand for B and to a lesser extent for Zn, and consequently, vegetables showed visual symptoms of B deficiency. Boron concentrations in dry biomass were below the critical limits with <10 mg B kg^{-1} in wheat, <21 mg B kg^{-1} in cauliflower and <23 B mg kg^{-1} in tomato. Soils in larger parts of Nepal are low in available B and that the ongoing system shifts increase in the demand for B and Zn in the currently emerging and more diversified production systems.

3.1 Introduction

Since the early 1980s, the rice-wheat annual double cropping has become the most important cropping system in the sub-tropical zone of South and South East Asia (Hobbs and Gupta 2003), where it substantially contributes to regional food and nutritional security and self-sufficiency (Gupta and Seth 2007). This cropping system covers an estimated 13.6 Mio ha in Indo-Gangetic Plains of India, Bangladesh, Nepal and Pakistan (Ladha et al. 2003) and around 18 Mio ha in China (Jiang et al. 2018). In this system, rice is cultivated during the warm monsoon season between June and October, while wheat is consequently grown in the cool, dry season between November and March (Hobbs and Gupta 2003; Gathala et al. 2013).

In recent years, however, the productivity and profitability of the rice-wheat rotations is declining throughout South Asia. Several drivers were identified for this tendency in the growing economic constraints in these rice-wheat systems. For instance, the relatively low market price for coarse grains (Jat et al. 2014), but also labor costs for crop establishment, weed control and harvest operations have substantially risen (Bhatt et al. 2016). In addition, the accessibility and provision of irrigation water has grown increasingly difficult, under concurrently declining groundwater tables (Ladha et al. 2007). Finally, crop yields have continuously declined in some areas, mainly associated with gradual declining soil fertility (Gami et al. 2001).

The emerging physical and economic water shortages have in turn triggered a (partial) substitution of irrigated lowland rice by the less water-demanding maize crop in the wet season (Ghimire et al. 2015), especially in areas where irrigation water is insufficient or unavailable in a timely manner. In addition, demographic growth, urbanization and associated changes in consumer preferences have gradually driven the substitution of wheat by higher-value crops such as potatoes and vegetables (Pokhrel and Soni 2017). Such changes are particularly prominent in the mid-hills and lowland areas of Nepal where agricultural production is gradually intensifying and increasingly shifting from subsistence to market-orientated production (Tiwari et al. 2008). In such situations, vegetable-based systems are seen to not only generate additional income for the rural farming communities but are also increasingly recognized as a means to improve human health (Raut et al. 2010).

Despite the changes in crop rotations, soils in the foothills of the central and eastern Himalayas and the Indo-Gangetic Plains are known for their low and declining fertility (Regmi et al. 2002; Ladha et al. 2003) and currently most soils are characterized by low inherent stocks and availability of the micronutrients such as Zn and B (Nadeem and Farooq 2019). A shift in the soil aeration status during the wet season due to the change from anaerobic (under lowland cultivation) to aerobic (during maize cropping) condition that is likely to further exacerbate the on-going soil fertility decline, mainly mirrored in a decline in soil carbon (C) and nitrogen (N) (Lal 2015). Furthermore, the newly emerging diversified vegetable-based rotations are likely to alter both the soil supply and crop demand for micronutrients (Rosen and Eliason 2005). The extent of such changes is likely to differ between sites due to e.g. climatic conditions and soil properties. Crop species and its production intensity further contribute to the more diverse demand for (micro-) nutrients.

Thus, we analyzed the soil nutrient content and the Zn and B availabilities under wet season rice and maize cropping, as well as their crop yields and the nutrient demand of wheat in comparison to cauliflower and tomato during the cold dry season. Therefore, biomass accumulation, economic important yields, and micronutrient uptake (here Zn and B) were comparatively assessed under both greenhouse conditions and at two field sites in the mid-hills and the lowland of Nepal.

3.2 Material and methods

3.2.1 Study sites

Geographical location: Pot experiments were conducted in a greenhouse in Kathmandu and the field trials were conducted at two field sites with contrasting edaphic and climatic conditions representing the mid-hills and the lowland of Nepal representing concurrently soils after long-term land uses with rice-wheat rotations. The study sites have been described details in section 2.1.1.

Climatic conditions: The climate in the rain-sheltered greenhouse, Kathmandu and the mid-hills and lowland field experimental site is previously reported in Chapter 2 (sections 2.2).

Land use: Both field experimental sites had been previously cultivated for more than one decade under rice-wheat rotation. The details of land use of the study sites have been explained in Chapter 2 (section 2.4).

Soil attributes: According to the World Reference Base (FAO 2014), the soils at the experimental sites are classified as Acrisols in the mid-hills and as Fluvisols in the lowland. The details soil attributes of study sites have been described in Chapter 2 (section 2.3).

3.2.2 Origin and attributes of plant materials

Wet season crops: The plant materials used in the wet season and their details have been presented in Chapter 2 (section 2.6.1).

Dry season crops: The plant materials used in the dry season and their attributes have been reported in Chapter 2 (section 2.6.2).

3.2.3 Experimental design and treatments application

The aim of the experiment was to study the implications of the emerging cropping systems in Nepal with maize replacing irrigated lowland rice and vegetables replacing wheat on soil attribute changes, crop performance attributes and the crops' B / Zn demands and contents. The experiment thus addressed cropping systems, which included crop types and their sequential arrangements and timing (Figure 2.4) and were applied in both the field and the greenhouse studies. Treatments comprised furthermore the two common production systems (rice vs. maize as wet season crops) and three dry season crops, (conventional wheat vs. tomato or cauliflower). Therefore 2 x 3-factorial experiments were laid out with four replications in a split-plot design at both field sites (see Appendix A1) whilst a completely randomized design was used in the greenhouse.

Crop management: The field-grown pre-crops of rice and maize in the wet season of 2017 were established and managed according to national recommendations. The aim was to reach a relative homogenization of site conditions appropriate for assessing pre-crop effects on performance attributes of the subsequently grown dry season crops. The rice seedbeds were prepared nearby the experimental sites and 24 days old seedlings were manually transplanted on June 20 into the hand tractor-plowed and wet-puddled soil at a 20 x 20 cm spacing using two seedlings per hill. Maize was dibble-seeded into the moist, aerobic soil at 60 x 20 cm spacing. Basal P and K fertilizers were applied as Single Super Phosphate and KCL at rates of 12 P kg and 21 kg K ha^{-1} respectively and mixed into the topsoil during land preparation. Nitrogen fertilizer was applied as urea at 100 kg N ha^{-1} in three equal splits dose, at 20 days after seeding/transplanting and at the panicle initiation (rice) and tasseling stages (maize). Rice plots were maintained flooded by well-irrigation until three weeks before harvest, while maize grew in an aerobic soil. All crops were harvested between late September (maize) and late October to mid-November 2017 (rice), by removing all aboveground biomass before land preparation consisting of plowing and harrowing for the wheat and vegetable crops.

Greenhouse experiment: For greenhouse experiments, about 1 t of topsoil (0 - 20 cm) representing each of the four field study sites/production systems was collected before the establishment of the wet season crops in 2017 and translocated to Kathmandu. Soils were dried, ground, and 8.8 kg soil filled into a total of 48, 10-L plastic pots with 18 cm depth and a surface of 500 cm^2 (2 sites x 2 systems, x 3 dry season crops x 4 replications = 48 pots). Soil moisture of all pots was maintained at approximately 70% of field capacities by weight-adjusted irrigation by adding demineralized water three times per week (Passioura 2006). The test crops wheat, cauliflower and tomato were seeded on 16 October 2017 to assess biomass and yield responses to the different preceding wet season crops and to identify their Zn and B uptake. The crop-specific recommended NPK fertilizer applications were used with 100 kg N ha^{-1} (as urea) and 21 kg K (KCl) ha^{-1} for wheat, and 200 kg N ha^{-1} and 67 and 83 kg K ha^{-1} for cauliflower and tomato, respectively. The applied recommended P rate used in all crops was 12.5 kg P ha^{-1} as Single Super Phosphate. One-third of the recommended N-dose and full doses of P and K, were applied as basal dressing and manually mixed into the potted soil, while the remaining N rates were surface-applied in two equal splits at 20 and 40 days after sowing. Crops were sown at a density of 28 wheat seeds per pot and nine seeds of tomato and cauliflower that were subsequently thinned (10 days after seedling) to three plants per pot. The total aboveground biomass was harvested at the end of January (vegetables) and early March (wheat). The economic yield was determined for wheat grain at 12% moisture, as well as for fresh curd (cauliflower) and fruit (tomato) weights.

Field experiment: After field homogenization by applying comparable management practices to both wet season crops, the field experiment for assessing performance attributes of the dry season crops (wheat vs. vegetables) was implemented at both the mid-hill and the lowland sites. Within each pre-crop treatment (rice vs. maize) sub-plots were established for the three subplot treatments in four replications. Plot dimensions were 3 x 6 m in the lowland but varied in the mid-hills depending on terrace widths from 3.0 x 3.5 m in the rice-based system plots and between 2.7 x 3.2 m (cauliflower) and 2.7 x 4.2 m (tomato) in the maize-based plots. While wheat was row-seeded at a rate of 120 kg ha^{-1} and row distances of 20 cm, vegetables were transplanted at 60 x 45 cm spacing. After seeding, all crops received initial irrigation of 3.7 L m^{-2} for homogenous germination and stand establishment. Subsequent irrigation events were applied based on deficit calculations using the software package CROPWAT (version 8). The FAO publication 56 on irrigation and drainage (Allen et al. 1998) was used as the reference for the calculation of the required amount of irrigation water in the field. Total aboveground biomass accumulation was determined at the early reproductive growth stages (90 and 60-70 days after seeding for wheat and vegetables, respectively) from 1 m^2 sub-plots used as representative harvest areas (wheat) or from 3 adjacent individual plants (vegetables), between late February and early April, depending on sites, systems and crops. The economic yields, determined as wheat grain at 12 % moisture were based on 1 x 1 m harvest areas and as fresh curd or fruits, for vegetables. While in cauliflower (before full bloom) one single harvest occurred at physiological maturity, the harvest of tomatoes extended over a 6’ week maturation interval and the total yield represents the cumulative fresh fruit weight collected during this period.

3.2.4 Soil and plant analyses

Soil samples: Initial composite topsoil samples (0-20 cm) were collected for baseline soil information of the study sites and production systems. The details of soil analysis were described in Chapter 2 (section 2.3). In all cases, the soil Zn content was close to or slightly above the critical limit for rice growth (Dobermann and Fairhurst 2000) however soil B content was always below the critical concentration of 0.5 mg B kg^{-1} (Sah and Brown 1997). The physical and chemical properties of the soils are given in Table 2.2.

Plant samples: Plants collected from both the greenhouse and the field trials at the early reproductive growth stage. The details of the methodology of plant sample analysis has been described in Chapter 2 (section 2.8). Boron concentrations in dry biomass were below the critical limits with 7 mg B kg^{-1} for wheat (Rashid et al. 2002), 26 mg B kg^{-1} for cauliflower (Batabyal et al. 2015) and of 20 mg B kg^{-1} for tomato (Rosen and Eliason 2005). Similarly, Zn content in wheat was above the critical limit of 20 mg Zn kg^{-1} at the mid-hill sites, and around the critical limit at the lowland site (Cakmak et al. 1996).

3.2.5 Statistical analysis

Data were subjected to an analysis of variance using the Package “Statistical Tool for Agricultural Research – STAR” (version 2.01). Descriptive statistics were calculated, and performance variability was assessed by standard errors of means. The Least Significant Difference (LSD) test was used for statistical mean separation, where appropriate.

3.3 Results

3.3.1 Soil attributes

The two experimental sites in the mid-hills and the lowland of Nepal are characterized by soils typical for these regions. Thus, acid Acrisols (pH 4.3) with sandy clay loam texture dominates the mid-hills areas of central and eastern Nepal, while sandy loam Fluvisols prevail in much of the lowland in the Terai (Table 2.2). The ongoing system changes mirrored in changes from wet-season lowland rice (anaerobic conditions) to the less water-demanding upland crop (aerobic condition) have resulted in a decline of total soil C from 1.8% C under the rice to 1.3% C under maize and observed a concomitant decline in soil N from 0.16% N under the rice to approximately 0.11% N under maize. The soil available Zn content was generally lower in the Fluvisols (mean of 2.1 Zn mg kg^{-1}) than in the Acrisols (mean of 2.7 Zn mg kg^{-1}) and was consistently lower under rice than under maize cultivation. No clear trends regarding the effects of sites or production systems were apparent, and $CaCl_2$ extractable B was lowest in the rice-based system in the lowland with 0.14 mg B kg^{-1} soil, and highest in the maize-based system in the mid-hills with 0.30 mg B kg^{-1} soil.

3.3.2 Crop response in the greenhouse

<u>Biomass accumulation:</u> The dry biomass accumulation at the early reproductive growth stage varied widely between plant species, soils and production systems (Table 3.1) ranging from 2.34-7.35 g dry matter pot^{-1}. The biomass accumulation tended to be lower in soils from the maize- than the rice-based systems and that of wheat was generally lower (2.34-4.33 g pot^{-1}) than that of the vegetable crops (2.06-7.35 g pot^{-1}). A wet season pre-crop of maize negatively affected the biomass accumulation of wheat in the mid-hills and of cauliflower and tomato in the lowland ($p<0.05$).

Table 3.1: Dry biomass accumulation and uptake of B and Zn at the early reproductive growth stage by wheat, cauliflower and tomato grown in potted soil from two production systems (rice vs maize as preceding wet season crop) in the mid-hills and lowland (greenhouse experiment, Nepal, 2017).

Sites	System#	DM (g pot $^{-1}$)			B uptake (mg pot $^{-1}$)			Zn uptake (mg pot $^{-1}$)		
		Wheat	Caulifl	Tomato	Wheat	Caulifl	Tomato	Wheat	Caulifl	Tomato
Mid-hills	Rice	4.33 a	4.13 ab	6.37 ab	0.03 a	0.05 b	0.07 b	0.20 a	0.30 a	0.37 b
	Maize	2.34 b	3.33 b	7.35 ab	0.01 c	0.05 b	0.09 a	0.11 a	0.31 a	0.50 a
Lowland	Rice	2.67 b	5.20 ab	6.02 ab	0.02 b	0.11 a	0.09 a	0.06 b	0.22 b	0.30 b
	Maize	2.45 b	2.06 b	2.13 c	0.02 b	0.04 b	0.02 c	0.05 b	0.10 b	0.10 c
Sites		ns	ns	**	ns	**	**	**	**	**
System		ns	**	ns	ns	**	**	**	ns	ns
Sites xSystem		ns	*	**	ns	**	**	*	ns	**

Means in one column with the same letter are not significantly different from each other (P>0.05 ANOVA followed by LSD test), #Preceding crop grown during the wet season, ns = non-significant, * significant at p<0.05 and ** significant at p<0.01. B concentration in the dry biomass of wheat 6-7 mg B kg^{-1}, of cauliflower 12-21 mg B kg^{-1}, and of tomato 11-15 mg B kg^{-1}. Zn concentration the dry biomass of wheat 19-47mg Zn kg^{-1}, of cauliflower 43-92 mg Zn kg^{-1}, and tomato 46-58 mg Zn kg^{-1}.

<u>Nutrient content:</u> The B concentration in the dry biomass was <7 mg B kg^{-1} DW in wheat, and 11-21 mg B kg^{-1} in cauliflower and tomato (Figure 3.1). These values were in all cases below the critical limits for crop growth. Zinc concentrations in the dry biomass were generally lower in the lowland compared to the mid-hill sites and tended to be higher following a crop of maize than of rice. With 47 mg Zn kg^{-1}, the Zn content in wheat was above the critical limit of 20 mg Zn kg^{-1} in soils from the mid-hill sites, but with 19-23 kg Zn kg^{-1} around the critical limit in soils from the lowland site. On the other hand, Zn contents in vegetables were above the critical tissue concentration in soils from the mid-hills and slightly below in soils from the lowland, irrespective of the production system (Figure 3.1). The crop uptake of B and Zn reflected the trend observed with biomass accumulation, ranging from 0.01-0.11 mg B pot^{-1} and from 0.05-0.50 mg Zn pot^{-1} (Table 3.1). It was always lower in wheat (0.01-0.03 mg B and 0.05-0.20 mg Zn pot^{-1}) compared to the vegetables (0.02-0.11 mg B and 0.10- 0.50 mg Zn pot^{-1}), and tended to be lower following a wet season crop of maize compared to rice.

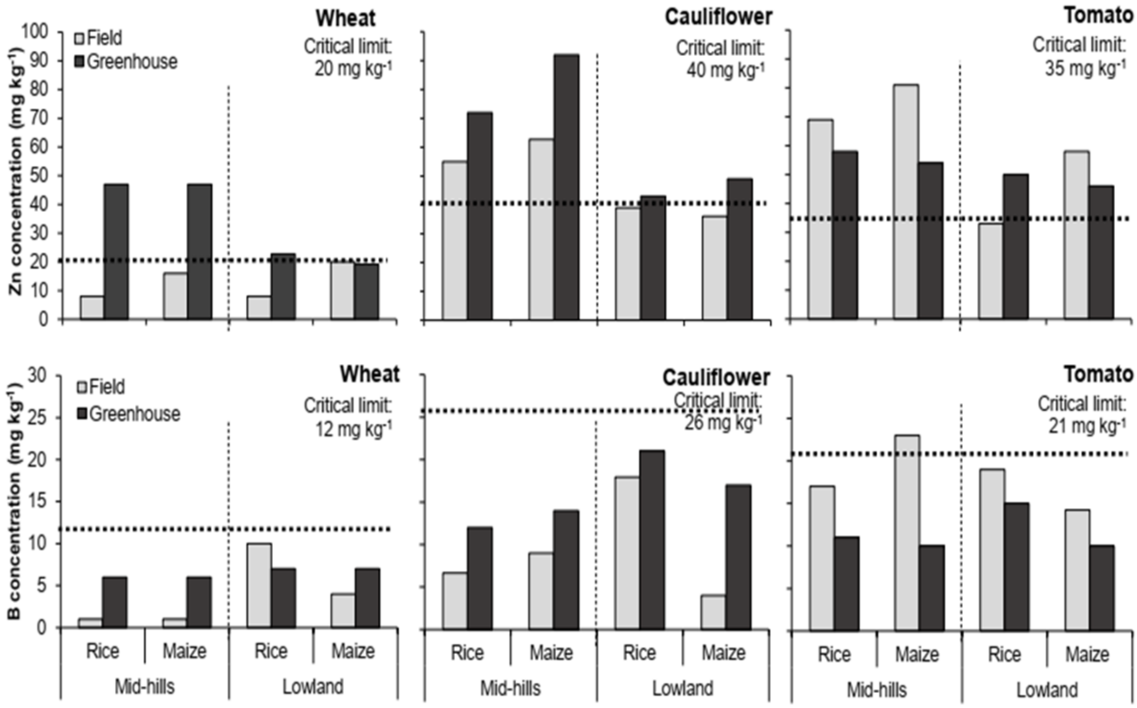

Figure 3.1: Effect of the study site (mid-hills vs. lowland) and preceding wet season crop (lowland rice vs. upland maize) in the field of the origin of the soil (greenhouse) on Zn and B concentrations in the dry biomass of wheat, cauliflower and tomato in Nepal.

Economic yield: The economic yield, comprising wheat grains at 12% moisture and fresh curd or fruit weights in vegetables at harvest, varied widely between plant species, soils and production systems (Table 3.2) ranging from 1.7-100.1 g harvested product pot^{-1}. Grain yields of wheat (1.7-3.0 g pot^{-1}) were much lower than fresh curd or fruit yields of the vegetables (13.9-100.1 g pot^{-1}) and tended to be higher in the lowland compared to the mid-hill soils. A wet season pre-crop of maize negatively affected the economic yield of wheat (significant in both soils types at $P< 0.05$) and of cauliflower (significant, at $P<0.05$ only in the soil from the lowland site). The tomato crop showed no apparent response to either site or production system. While no significant interactions of site and system were observed for dry matter accumulation, B uptake and economic yield of wheat, such interactions were however significant for dry matter, economic yields, and B uptake by cauliflower at $p<0.05$. In the case of tomato, sites and systems interactions were significant for dry biomass accumulation Zn and B uptake at $p<0.01$.

3.3.3 Crop response in the field

The field trials could validate in part the trends observed in the greenhouse experiment. The highest yield of the wheat grain of 6.1 t ha^{-1} was obtained following a crop of rice at the mid-hills site while the lowest yield of 1.0 t ha^{-1} was observed in the lowland site (Table 3.2). While the production system had no economic effect on yields of either tomato or cauliflower at the lowland site, yields were significantly higher following maize compared to rice as a preceding wet season crop, at the mid-hills site, with a maximum of 13.0 t ha^{-1} of fresh cauliflower and 16.8 t ha^{-1} of fresh tomato (Table 3.2).

Table 3.2: Effect of the study site and preceding wet season crop (lowland rice vs. maize) in the field or of the origin of the soil (greenhouse) on economic yield of wheat grain at 12% moisture and of fresh curd/fruit of cauliflower and tomato (greenhouse experiment in potted soil in Kathmandu and field experiments at Rampur and Kavre, Nepal, 2017).

Sites	System#	Economic yield					
		Pot experiment (g pot $^{-1}$)			Field experiment (t ha^{-1})		
		Wheat	Caulifl	Tomato	Wheat	Caulifl	Tomato
Mid-hills	Rice	2.0 b	18.3 b	29.2 c	6.1 a	0.3¤	10.7 ab
	Maize	1.7 b	35.4 ab	100.1 a	5.2 ab	13.0 a	16.8 a
Lowland	Rice	3.0 a	60.6 a	79.4 b	1.0¤ c	1.5 b	6.7 b
	Maize	2.0 b	13.9 bc	60.1 b	4.3 b	1.0 b	7.4 b
Sites		*	ns	ns	**	**	*
System		*	**	ns	ns	**	ns
Sites x System		ns	**	ns	**	**	ns

Means in one column with the same letter are not significantly different from each other (P>0.05 ANOVA followed by LSD test) and different letters within a column denote significant differences at $P < 0.05$ (Least Significant Difference test).

#Preceding crop grown during the wet season
¤ low yield due to late planting response to high temperature during the early vegetative growth stage
ns = non-significant, * significant at $p<0.05$ and ** significant at $p<0.01$.

The B concentration in tissues of field-grown crops was below the critical limit (<23 mg B kg^{-1}). Plant tissue B concentrations of tomato were higher while those of wheat and cauliflower were lower than in the greenhouse experiment (Figure 3.1).The Zn concentrations largely followed the trends observed from the greenhouse trial except for field-grown wheat, where Zn levels were below the critical limit, irrespective of site and production system. Total nutrient uptake was highly variable with 55 - 350 g B ha^{-1} of B and about 400 - 900 g Zn ha^{-1} (data not shown).

3.4 Discussion

3.4.1 Effects of replacing rice by maize-based cropping system on soil attributes

The ongoing system changes from wet-season lowland rice (anaerobic conditions) to less water-demanding upland maize (aerobic conditions) reduced soil C and N contents already after 3-5 years after the system shift. It has been shown before that soil submergence can contribute to conserving soil C (Kirk 2004), while soil aerobic crop production does accelerate soil microbial mineralization processes (Lal 2015). Consequently, the ongoing shift from wet-season lowland rice to upland maize is likely to exacerbate the degradation of the already poor soils (He et al. 2017), prevailing in much of Nepal (Gami et al. 2001).

Zn is an essential micronutrient involved in a wide variety of plant physiological processes, including photochemical reactions, carbonic anhydrase activity, the biosynthesis of chlorophyll and cell membrane integrity (Tsonev and Lidon 2012). The content of plant-available Zn in soils of Nepal is generally low (Hafeez 2013) and lays within or only slightly above the reported deficiency limit of 2 mg Zn kg^{-1} soil (Dobermann and Fairhurst 2000). The lower soil Zn levels observed in the lowland (mean of 2.1 mg Zn kg^{-1} soil) compared to the mid-hills (mean of 2.7 mg kg^{-1}) and consistently lower levels under rice than under maize. With Zn uptake by rice largely exceeding that of maize (Wissuwa et al. 2008), the emerging production system shifts may thus reduce the removal of Zn from the inherently deficient soils and contribute to reducing the demand for Zn fertilizers.

Plants require B for cross-linking pectins in the cell wall and middle lamella, and many physiological processes such as division of meristematic cells, membrane integrity, phenol metabolism or plant reproduction are impaired under conditions of deficient B supply (Brown et al. 2002). The plant-available soil B content (here $CaCl_2$-

extractable B of 0.1-0.3 mg kg^{-1}) was always below the critical level of 0.5 mg kg^{-1} soil determined for cereals (Gupta 2007), groundnuts (Mahendran et al. 2016), vegetables (Shorrocks 1997), and oilseed rape (Xue et al. 1998), irrespective of the site and the production system. However, lowest soil B contents were observed in the irrigated lowland rice-based systems, where leaching of B from the acidic and sandy soils is likely to further exacerbate an already critically low B status (Saleem et al. 2011). Tripathi & Jones (2013) also reported low levels of soil B availability in lowland rice-based systems in Nepal. It can be concluded that the currently ongoing and future expected changes towards intensified and diversified food crop production systems in Nepal will differentially affect crop nutrient demands. While a shift towards upland maize may reduce Zn removal in the future, neither the current nor the emerging crop production systems are likely to be sustained without additions of Zn and B by mineral or organic fertilizer sources.

3.4.2 Effect of micronutrients availability on dry season crop response

Due to the low soil contents of available B and Zn, the biomass accumulation and economic yields of dry season crops were generally low, particularly at the lowland site. In the mid-hills, grain yields of field-grown wheat could reach 5.2-6.1 t ha^{-1}, which was significantly higher than in the lowland. This partially contradicts the assumption that soils in the mid-hills of Nepal are not suitable for wheat production due to low soil pH, low C and N and low available Zn contents (Heider et al. 2018). Possibly, climatic conditions with colder winters and transition seasons in the mid-hills favor wheat growth compared to the hotter temperatures in the lowland. A pre-crop of maize reduced soil N contents, and despite an application of 100 kg mineral N ha^{-1}, wheat yields were considerably lower after maize than after rice in the greenhouse experiment. In the field, however, such trends were less apparent.

The mid-hills probably provided more favourable climatic conditions than the lowland for growing temperate crops such as wheat, cauliflower, and tomato, irrespective of the preceding wet season crop. Irrespective of the site or the production system, the economic yields of cauliflower and tomato were largely below the reported potential of 30-42 t ha^{-1} of fresh cauliflower curd (Giri et al. 2018) and 17-25 t ha^{-1} for tomato (Shrestha and Sah 2015). National yield standards are 14 and 17 t ha^{-1} for cauliflower and tomato, respectively (VDD 2015). Besides an unfavourable temperature regime at the lowland site (cauliflower after rice), the severe soil B deficiency, and possible the low Zn availability are likely the main culprits for the observed low yields.

Zn and B are reportedly the most deficient micronutrients in agricultural soils of Nepal (Andersen 2007) and widely limit crop yields (Karki et al. 2005). Compared to cereal crops, i.e. the conventional rice-wheat rotation, vegetable crops require substantially more Zn and B (Wimmer et al. 2015). The B concentrations in plant tissues were always below the crop-specific critical limits of <23 mg B kg^{-1} (Figure 3.1). Without B amendments tissue B concentration in cauliflower and tomato were below the critical limits of 26 mg B kg^{-1} of B for cauliflower (Batabyal et al. 2015), and of 20 mg B kg^{-1} for tomato (Rosen and Eliason 2005) in both greenhouse and field experiments. The resulting B, and to a lesser degree Zn deficiencies adversely affects yields as monitored as well in this experiment (Ozturk et al. 2010), marketability of the harvested product (Tombuloglu et al. 2012), and possibly the nutritional quality (Ozturk et al. 2010; Tombuloglu et al. 2012; Liu et al. 2019). Thus the addition of Zn and B has been shown to enhance the product quality of tomato in Turkey (Sathya et al. 2013; Abo-Hamad and El-Feky 2014) and cauliflower (Gupta 2007) in B and Zn deficient soils. Particularly B deficiency can adversely affect tomato flowering and fruiting, reducing not only yield but particularly the product quality (Bele and Thakur 2019). In the present study, visual Zn deficiency symptoms were observed primarily during the early growth stages of wheat at the lowland site, particularly with rice as the preceding crop, showing typical inter-coastal chlorosis with small necrotic spots on the youngest leaves. On the other hand, B deficiency occurred in both vegetable crops, irrespective of the site and system, with brittle petioles and small fruits with chapped surfaces and brown necrotic areas on the of tomato and hollow stem with brownish necrosis as well as dark-brown spots on the surface of the cauliflower curd, reducing substantially the suitability for their marketing.

3.5 Conclusions

In the inherently B- and to a lesser extent Zn- deficient soils in larger regions of Nepal, and ongoing system shifts differentially affect soil nutrient availability and substantially increase the demand of Zn and B in the emerging and diversified production systems. Changing the soil aeration status in the wet season from submerged / anaerobic (irrigated lowland rice) to aerobic (upland maize) entailed declining soil C and N contents, while available soil Zn tended to increase. Vegetables replacing wheat as dry season crops have a much higher demand for B and Zn, which is currently not met by soil supply, and will require external micronutrient application in the future.

Chapter 4

Based on the journal article:

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4 BORON AND ZINC FERTILIZER APPLICATIONS ARE ESSENTIAL IN EMERGING VEGETABLE-BASED CROP ROTATIONS IN NEPAL

Abstract

Since recently, the traditional rice-wheat rotation systems in Nepal are subject to drastic changes. Progressing urbanisation and shifting consumer preferences drive a replacement of wheat by high-value vegetables during the cold dry season, while emerging water shortages prevent permanent soil flooding during the monsoon season, leading to partial substitution of lowland rice by less water-consuming upland crops. Associated changes in soil aeration status affect soil nutrient availability while particularly vegetables enhance the demand for the critically limiting micronutrients B and Zn. In both rice- (anaerobic) and maize-based (aerobic) systems thus assessed the differential response of traditional winter wheat in comparison to cauliflower and tomato to applied B and Zn fertilizers. An experiment conducted (i) in a pot trial with two contrasting soil types (Acrisols vs. Fluvisols) and (ii) in field validation trials at two contrasting sites (representing lowland vs. mid-hills) in Nepal. The on-going shift from flooded rice to aerobic maize during the wet season negatively affected dry matter accumulation and grain yield of the dry season wheat, but not of cauliflower and tomato. While Zn application tended to increase wheat yields under field conditions, B application induced no significant effect, irrespective of the soil or production site. However, low to moderate applications of B (2.0-4.4 kg ha^{-1}) and Zn (3.3-4.4 kg ha^{-1}) nearly doubled biomass accumulation and nutrient uptake of vegetables and increased the economic yields of cauliflower and tomato between 8 and >100%. These responses were generally more pronounced in the Fluvisols than the Acrisols. While overall yields of wheat and temperate vegetables were higher in the cool mid-hills the relative yield responses to applied B were more pronounced in the lowland than the mid-hill sites. On average, the benefit-cost-ratio or partial factor productivities of applied fertilizer were low to moderate in wheat, with 1 and 8 € increase in net revenue per € of investment in B and Zn, respectively. In the vegetables, this partial factor productivity increased to about 4 € $€^{-1}$ investment with Zn and reached about 43€ $€^{-1}$ investment in B, irrespective of the production site. While the application of Zn fertilizers can thus moderately improve the performance of traditional rice-wheat rotations, B and to a lesser extent Zn application become essential and highly profitable when shifting towards vegetable cropping. The demand for B and Zn fertilizers is foreseen to dramatically increase with progressing urbanisation and the associated shifts in production systems of Nepal.

4.1 Introduction

The annual rice-wheat double-crop rotation is the single most important crop production system in the Indo-Gangetic Plains (IGP) including the Himalayan foothills. Outside of South Asia, rice-wheat rotations cover an estimated 30 Mio ha in South and Central China (Nie and Peng 2017), but there the area is in the process of declining (FAO 2020). Several recent trends and change processes exert pressure on the conventional rice-wheat rotations and resulted in modifications of crops and agricultural practices. Most prominent in shaping new production systems in the region are (1) emerging water shortages and (2) economic drivers. Particularly the highly water-demanding lowland rice production is increasingly challenged by water shortages, and alternative production strategies and systems are emerging (Chauhan et al. 2012). Thus, in some areas, farmers are shifting from irrigated lowland rice to rain-fed upland crops (i.e. maize). Alternatively, water-saving rice production technologies were introduced, which included aerobic rice (Bouman et al. 2005), alternate wetting and drying – AWD (Carrijo et al. 2017) the system of rice intensification – SRI (Karki 2010), and reduced tillage and mulching systems (Yadvinder-Singh et al. 2014). Progressing urbanization entails a shifting of rice-wheat cereal cropping system to market-oriented high-value vegetable production. While concurrently meeting the changing food demands by consumers in urban centers (Tiwari et al. 2010). However, the productivity of conventional rice-wheat rotations and emerging vegetable-based systems is challenged by widespread and severe soil deficiencies of B and Zn in the IGP region (Nadeem and Farooq 2019), including Nepal's mid-hills and lowland (Andersen 2002).

Zn deficiency affects most rice-growing areas globally (Alloway 2009), due to the crop's comparatively high Zn demand (Dobermann and Fairhurst 2000). However, the mid-hills and lowland areas of the Himalayas are particularly affected. There, Zn deficiency is one of the determinants for the reported productivity decline in rice-wheat systems (Nayyar et al. 2001), and affects nearly 50% of the rice field of Nepal. While B deficiency is reportedly a major constraint to crop production in some 80 countries (Sillanpää 1982), where about 132 crops are affected (Shorrocks 1997), the

Himalayan mid-hills and adjoining lowland areas are probably the world's largest area of B deficient soils (Rerkasem and Jamjod 2004). Boron deficiency causes sterility in wheat (Rerkasem and Jamjod 1997) and generally affects performance attributes of most non-graminaceous rotation crops (Rerkasem and Jamjod 2004). B is acknowledged as the second most limiting micronutrient in crop production of South Asia after Zn (Padbhushan and Kumar 2017) and both are widely recognized as major limiting factors for the productivity of rice-wheat cropping in IGP region (Akhtar et al. 2013).

This situation is likely to be further exacerbated by the on-going production system shifts. Thus, changes in soil aeration status that are resulting from less water-consuming cropping practices, enhance soil carbon mineralization and can stimulate losses of native soil N (Becker et al. 2007), and particularly affect the plant availability of phosphorus (Zhang et al. 2004) and most micronutrient metals, including Zn (Rengel 2015). In addition, soil acidification associated with aerobic conditions combined with soil acidity will result in the pH-dependent transformation of borate into boric acid (Goldbach 2020), which is easily removed by leaching with high rainfall intensities and in highly percolating sandy soils (Shaaban 2010), conditions that prevail in much of the mid-hills and the lowland of the IGP. In addition, the amounts of P and most micronutrients removed with crop production are much higher with vegetables than with wheat (Hu et al. 1996; Haytova 2013).

Thus, combined with the aerobic soil status that is associated with water-saving wet season cropping, the increased nutrient removal by dry season vegetables are hypothesized to aggravate the prevailing Zn and B deficiencies. This problem can be eased only through the application of site- and system-specific rates of Zn and B fertilizers. By sustaining the emerging production systems, these measures can hence contribute to income generation and regional food security. To test this hypothesis, the following objectives were addressed in a pot trial with two contrasting soil types (Acrisols vs. Fluvisols) and at two contrasting field sites (mid-hills vs. lowland) in Nepal:

(1) Assess soil attribute changes associated with the emerging cropping practices with respects to nutrient availability.

(2) Determine the effects of Zn and B fertilizer application on dry matter accumulation and nutrient content of dry season crops such as wheat, cauliflower, and tomato.

(3) Quantify the responsiveness of yields and economic benefits to applied Zn and B fertilizers in wheat, cauliflower, and tomato.

4.2 Material and methods

4.2.1 Study sites

Geographical location: A pot experiment was conducted in a rain-sheltered greenhouse in Kathmandu and findings were validated under field conditions at two field sites with contrasting edaphic and climatic conditions in the mid-hills and the lowland of Nepal. For details of the study sites were described in Chapter 2 (section 2.1.1).

Soils: Topsoil (0-20 cm) samples were collected at the onset of the wet season in 2017 and analyzed for texture, pH (H_2O), total C and N, available Zn and extractable B. The details of soil analysis were reported in Chapter 2 (section 2.3). In all cases, the soil Zn content was close to or slightly above the critical limit for rice growth of 2 mg Zn kg^{-1} (Dobermann and Fairhurst 2000). In addition, the available soil B content was below 0.3 mg B kg^{-1} for all soils / sites, and is thus below the marginal concentration for B sensitive crops of 1 mg B kg^{-1} (Gupta 2007).

Climatic conditions: The climatic conditions in the rain-shed greenhouse (monthly air humidity and mean minimum and maximum temperatures in the greenhouse – 2017) and at the field sites (long-term monthly averages of minimum and maximum temperatures and rainfall at the field sites – 2007-2018) are presented in Chapter 2 (section 2.2).

Land use: The details of the land use and the cropping pattern has been visualized previously and was presented in Figure 2.4 in Chapter 2 (section 2.4).

4.2.2 Plant material and analyses

The details of the plant material (section 2.6) and method of B and Zn analysis (section 2.8) were described in Chapter 2.

4.2.3 Treatment application

Treatments imposed comprised two production systems (rice vs. maize as wet season crops), three dry season crops (wheat vs. tomato or cauliflower), and four fertilizer treatments (recommended NPK rate, NPK+Zn, NPK+B, and NPK+Zn+B). This amounted to the 2 (production systems) x 3 (dry season crops) x 4 (fertilizer treatments) factorial experiments, which were laid out with four replications in a split-plot design at both field sites. In the greenhouse, 192 pots were arranged in a completely randomized design (representing 2 soils x 2 systems x 3 crops x 4 fertilizer treatments x 4 replications).

Pot trials: For the pot experiment, dried, ground, and sieved topsoil (0-20 cm) samples from both field sites and production systems were filled at 8.8 kg soil per pot into 10 L free-draining PVC pots with 28 cm diameter. Soils were maintained at 70% field capacity by weekly weight-adjusted irrigation with demineralized water. The test crops were established on 16 October 2017 by seedlings of wheat (9 seedlings pot^{-1}), cauliflower and tomato (3 seedlings pot^{-1}). The national recommended crop-specific NPK fertilizer application rates were used, which amounted to 100 kg N ha^{-1} as urea and 20 kg K ha^{-1} as KCL for wheat, and 200 kg N ha^{-1} and 67 and 83 kg K ha^{-1} for cauliflower and tomato, respectively. In addition, 3.3 kg Zn ha^{-1} for wheat, 4.4 kg Zn ha^{-1} for tomato and cauliflower (as $ZnSO_4$), and 2.0 kg B ha^{-1} in wheat and 4.4 kg B ha^{-1} for cauliflower as well as 2.2 kg B ha^{-1} tomato applied. All pots received uniformly the recommended P application rate of 12.5 kg P ha^{-1} in the form of Single Super Phosphate. One-third of the recommended N-dose and full doses of P, K, B and Zn were applied as basal dressing and manually mixed into the potted soil before seedling, while the remaining N rates were top-dressed (surface-applied) at two equal splits at 20 and 40 days after seedling. The total above-ground biomass was harvested between end-January (vegetables) and early March (wheat). The economic yield was determined as wheat grain weight at 12% moisture content and as the fresh weight of cauliflower curd and tomato fruit at harvest.

Field trials: In the field experiments, the wet season pre-crops of rice or maize were homogenously established across all experimental field plots at both sites in June 2017 following national recommended establishment and NPK fertilizer rates. After crop harvest in November (maize) and December (rice), the field areas were parceled for treatment application. The dimensions of individual treatment plots were 3 x 6 m in the lowland and 3.0 x 3.5 m in the mid-hills. While wheat was row-seeded at a rate of 120 kg ha^{-1} at row distances of 20 cm, vegetables were transplanted at 60 x 45 cm spacing. Establishment dates differed between sites and growth durations of the pre-crops. After seeding/transplanting, all crops received initial irrigation of 3.7 L m^{-2} for homogenous germination and stand establishment. Subsequent irrigation events were applied based on soil moisture deficit calculations using the software package FAO CROPWAT (version 8). The crop specific recommended NPK, as well as the B and Zn fertilizer rates, were applied as indicated in the description of the pot experiment. Total aboveground biomass accumulation was determined at the early reproductive growth stages (90 and 60-70 days after seeding for wheat and vegetables, respectively) from 1 m^2 sub-plots used as representative harvest areas (wheat) or from 3 adjacent individual plants (vegetables), between late February and early April, depending on sites, systems and crops.

Calculations: The economic yields, determined as wheat grain weight at 12 % moisture and as fresh curd or fruits weight, for cauliflower and tomato. While in cauliflower (before full bloom) one single harvest occurred at physiological maturity, the harvest of tomatoes extended over a 6-week maturation interval and the total yield represents the cumulative fresh fruit weight collected during this period. The partial factor productivity measures the contribution of additional fertilizers to increase the crop yield and remaining other factors are constant which estimates to the efficiency of resources use and easy for the farmer to support the decision for new technology adaptation. The partial factor productivity of applied B and Zn fertilizers was based on current prices of borax and Zn-sulfate in the sub-region. There are no B and Zn fertilizers are currently available at rural markets in Nepal and on the commodity prices of wheat, cauliflower, and tomato on the study sites. The productivity was calculated as the monetary gain from yield increases due to the application of B and/or Zn divided by the crop-specific cost of B and Zn applications and expressed as Euro gain per Euro investment.

4.2.4 Statistical analysis

Data was analyzed using one-way analysis of variance in STAR (version 2.01) and mean difference were separated using LSD at 5% level of significance. The interaction between site, system, and crop analyzed using mix model in Stata 14.2. Mean is comparing by pairwise comparison between and within factors. B and Zn concentrations were presented graphically using bar charts in excel and their performance variability was assessed using standard errors of means.

4.3 Results

4.3.1 Soil attributes

The two experimental sites in the mid-hills and the lowland of Nepal are characterized by soils typical for these regions. Thus, acid Acrisols (pH 4.3) with sandy clay loam texture dominate the mid-hills areas of central and eastern Nepal, while sandy loam Fluvisols prevail in much of the lowland in the Terai (Table 2.2).The ongoing system changes mirrored in changes from wet season lowland rice (anaerobic conditions) to the less water-demanding upland crop (aerobic condition) have resulted in a decline of total soil C from 1.8% C under rice to 1.3% C under maize and observed a concomitant decline in soil N from 0.16% N under rice to approximately 0.11% N under maize. The soil available Zn content was generally lower in the Fluvisols (mean of 2.1 Zn mg kg^{-1}) than in the Acrisols (mean of 2.7 Zn mg kg^{-1}) and was consistently lower under rice than under maize cultivation. No clear trends regarding the effects of sites or production systems were apparent, and $CaCl_2$ extractable B was lowest in the rice-based system in the lowland with 0.14 mg B kg^{-1} soil, and highest in the maize-based system in the mid-hills with 0.30 mg B kg^{-1} soil.

4.3.2 Crop response to soil type, system change and applied Zn and B

The dry biomass accumulation of plants differed between crops and was significantly affected by site/soil type. Significant effects were also observed for production systems and fertilizer applications (Table 4.1). Under field conditions, dry biomass accumulation was highest at the mid-hills site (Acrisols) for wheat and the lowland site (Fluvisols) for vegetables. In the pot trial, this general effect was not observed, indicating that differences in growth were not related to the soil type, but rather to combinations of soil and climate conditions. However, in the greenhouse, the tomato grew better in the Acrisols compared to the Fluvisols. A change in the production system (shift from rice to maize or from flooded to aerobic soil) negatively affected all crops grown in potted soil apart from tomato in the Acrisols. These yield-reducing effects following maize were most pronounced in wheat and reflected the decline in soil C and N contents. However, this trend was not confirmed under field conditions, where biomass was either not affected in the maize system (Table 4.1).

The strongest responses in biomass accumulation to added B and Zn were observed in soils (at sites) with the lowest available B or Zn contents. Thus, wheat biomass responded most to added Zn under rice cultivation, and especially in the Fluvisols which had the lowest concentration of available Zn with <1.9 mg Zn kg^{-1} soil, while not being significantly improved by B fertilizer. On the other hand, vegetables responded more to added B compared to added Zn, and this was seen most in the lowland soil under maize cultivation with sandy soil with lowest concentration of water-extractable B. The application of B and Zn, sole or in combination generally increased the respective nutrient concentration in the biomass (Figure 4.1). Thus, Zn applications (with or without additional B) increased wheat tissue Zn contents from 34 to 41 mg Zn kg^{-1} in the greenhouse and from 13 to 22 mg Zn kg^{-1} at the field sites (the critical limit in wheat tissue is <16 mg Zn kg^{-1} (Cakmak et al. 1996). The concomitant effect on tissue Zn content of the vegetables was an increase from 50-65 mg Zn kg^{-1} in the NPK treatment to >80 mg Zn kg^{-1} in Zn-treated cauliflower, irrespective of soil or site (critical limit: 20 mg Zn kg^{-1}), and from 50-60 mg Zn kg^{-1} to 66 mg Zn kg^{-1} (pot) and >85 mg Zn kg^{-1} (field) for tomato (critical limit: 25 mg Zn kg^{-1}) (Hochmuth et al. 2015).

Table 4.1: Effect of mineral fertilizer application on the dry biomass production at the early reproductive growth stage of wheat, cauliflower and tomato grown in the greenhouse in Kathmandu and under field conditions in two production systems (rice vs. maize as preceding wet season crops), Nepal (2017).

Site/Soil	System#	Treatments	Dry matter (g m^{-2}) greenhouse			Dry matter (g m^{-2}) in the field		
			Wheat	Caulifl	Tomato	Wheat	Caulifl	Tomato
Mid-hills (Acrisols)	Rice	NPK	70	67	103	1275	51 c	23
		NPK+Zn	89 ns	74 ns	103 ns	1642 ns	96 c	28 ns
		NPK+B	74	65	95	1283	585 a	54
		NPK+Zn+B	84	63	93	1512	420 b	61
	Maize	NPK	38	54	152	1480	399	39
		NPK+Zn	36 ns	52 ns	147 ns	1525 ns	387 ns	39 ns
		NPK+B	48	59	141	1651	544	43
		NPK+Zn+B	38	60	130	1690	527	61
Lowland (Fluvisols)	Rice	NPK	43	84	98	188	506	61
		NPK+Zn	103 ns	76 ns	88 ns	233 ns	738 ns	63 ns
		NPK+B	70	92	85	234	836	73
		NPK+Zn+B	100	96	84	275	818	68
	Maize	NPK	40	34 b	35 b	429	163 b	55
		NPK+Zn	23 ns	73 a	42 b	478 ns	268 b	55 ns
		NPK+B	31	75 a	62 a	330	942 a	176
		NPK+Zn+B	31	80 a	49 ab	396	903 a	151
Site / Soil			ns	***	***	***	***	***
System			***	***	ns	*	ns	ns
Site / Soil x system			ns	ns	***	ns	**	ns
Treatment			ns	*	ns	ns	***	*
Site / Soil x treatment			ns	ns	ns	ns	ns	ns
System x treatment			*	ns	ns	ns	ns	ns
Site x system x treatment			ns	ns	ns	ns	***	ns

Preceding crop during the wet season; x interaction between factors. Data values followed by the different letters within a column are significantly different by Least Significant Difference test (LSD) at p<0.05; ns = non-significant; * significant at p<0.05; ** significant at p<0.01 and *** significant at p<0.001.

The application of B fertilizer increased tissue B contents from 5 to 22 mg B kg^{-1} (wheat), 12 to 75 mg B kg^{-1} (cauliflower) and 15 to 48 mg B kg^{-1} (tomato), with least responses observed in wheat and highest response in cauliflower (mean across the two soil types/production sites) (Figure 4.1). The combined response of biomass and tissue nutrient concentrations resulted in distinct B and Zn uptake of different crops at different sites or in different systems.

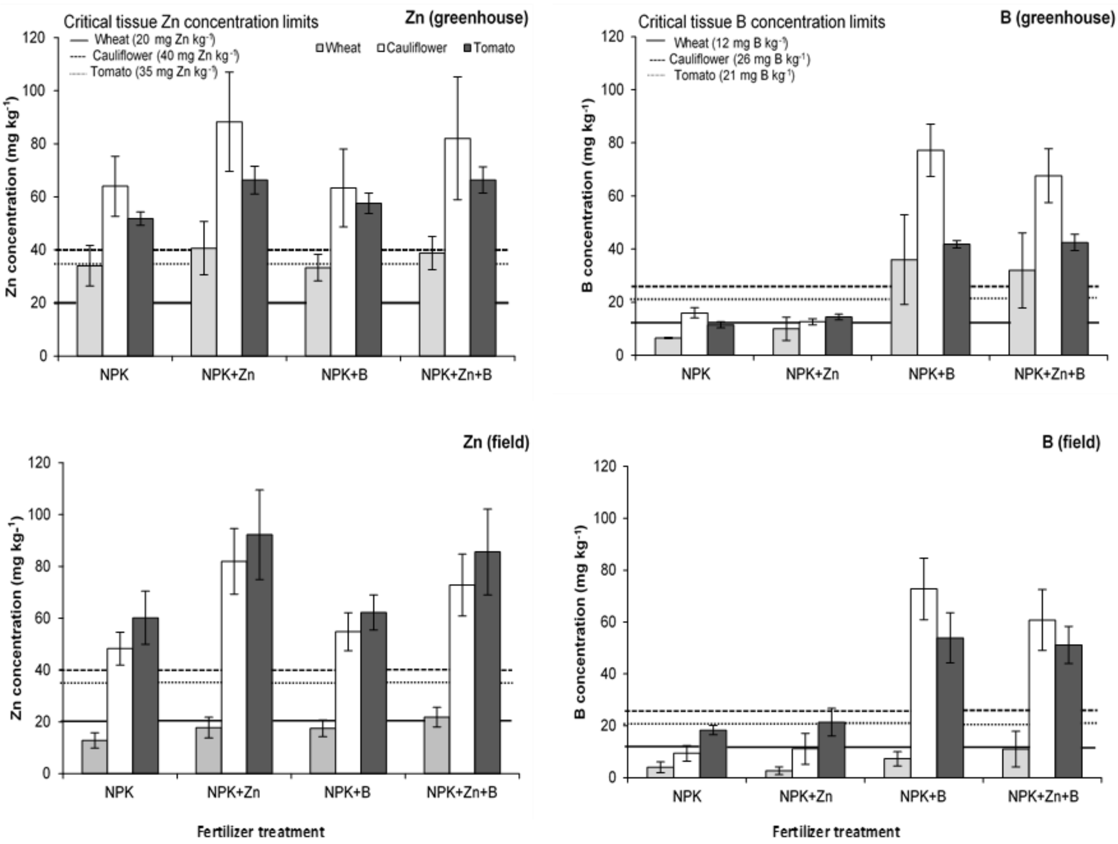


Figure 4.1: Effect of mineral fertilizer application on Zn and B concentration in the dry biomass at the early reproductive growth stage of wheat, cauliflower and tomato grown in the greenhouse in Kathmandu (upper) and under field conditions (lower graphs), Nepal (2017). Data present means of two field sites (mid-hills vs. lowland), two production systems (rice vs. maize as preceding wet season crops) and 4 replications (n=16).

The application of Zn corresponding to 330 mg Zn m^{-2} significantly increased the Zn uptake by wheat in the potted Fluvisols with a maximum Zn uptake by wheat of 47 mg Zn m^{-2} in the maize-based mid-hills field. For vegetables, the Zn uptake was significantly affected in potted soils only in the maize-based system. Under field conditions, the Zn uptake was significantly increased in the rice-based system of the mid-hills (cauliflower and tomato), and in the maize-based systems of the lowland (cauliflower), with a maximum of 77 mg Zn m^{-2} (cauliflower) and 11 mg Zn m^{-2} (tomato)

(Table 4.2). Application of B corresponding to 200 (wheat) or 440 (vegetables) mg B m^{-2} enhanced B uptake in all systems and sites and under both greenhouse and field conditions. Highest B uptake was always observed with cauliflower, reaching 8 mg B m^{-2} in the potted Fluvisols and 96 mg B m^{-2} at the lowland field site (Table 4.3).

4.3.3 Economic yield and partial factor productivity

Depending on the crop, the site/soil and the B / Zn application, the economic yields ranged from 31-2246 g m^{-2} in the greenhouse, and from 0,94 to 20,43 Mg ha^{-1} (corresponding to 94 - 2246 g m^{-2}) in the field (Figure 4.2). In the pot trial, economic yields of unfertilized wheat, cauliflower and tomato were not significantly different between Fluvisols and Acrisols (Figure 4.2) but tended to be higher when growing in the soil of the rice-based, compared to the maize-based system (data not shown). For vegetables, the yield increase by additional Zn (alone) was more pronounced in the Acrisols, while B (with or without Zn) application was more effective in the Fluvisols, especially for tomato.

Under field conditions, all crops yielded more at the relatively colder mid-hills than the warm subtropical lowland site (Figure 4.2), irrespective of being fertilized or not. The response to added Zn (without B) was also stronger in the mid-hills site compared to the lowland site but was overall relatively small with an economic yield gain of 0-95 g m^{-2} (wheat), 0-271 g m^{-2} (cauliflower) and 25-367 g m^{-2} (tomato). Addition of B fertilizer (with or without Zn) did not significantly improve the yield of wheat, irrespective of the site, but the yield of vegetables was strongly enhanced, with yield gains of 884-1104 g m^{-2} (cauliflower) and 532-1473 g m^{-2} (tomato).

Vegetable yields obtained with a combined application of B and Zn fertilizers were similar or higher than those obtained by B application alone, and increased economic yield of vegetables compared to NPK alone by 63% (tomato) to more than 59% (cauliflower) in the greenhouse, and by more than 100% in tomato and more than 200% in cauliflower under field conditions (see Appendix 4). This differential yield response to added B and Zn fertilizers would have resulted in economic benefits for farmers that are expressed here as the partial factor productivity or monetary gains from produce sale with B/Zn application in relation to fertilizer application costs. The application of borax at rates equivalent to 2.0 – 4.4 kg B ha^{-1} resulted in increased production that led at current market prices to monetary gains of 19 € ha^{-1} in wheat, but that increased to 871 – 1714 € ha^{-1} in vegetables. Mechanization is difficult in the mid-hills region and the cost of production is higher than lowland sites.

Table 4.2: Effect of mineral fertilizer application on zinc uptake at early reproductive stage of wheat, cauliflower and tomato grown in the greenhouse (Acrosols and Fluvisols) in Kathmandu and under field conditions (mid-hills and lowland), in two production systems (rice vs. maize as preceding wet season crops), Nepal (2017).

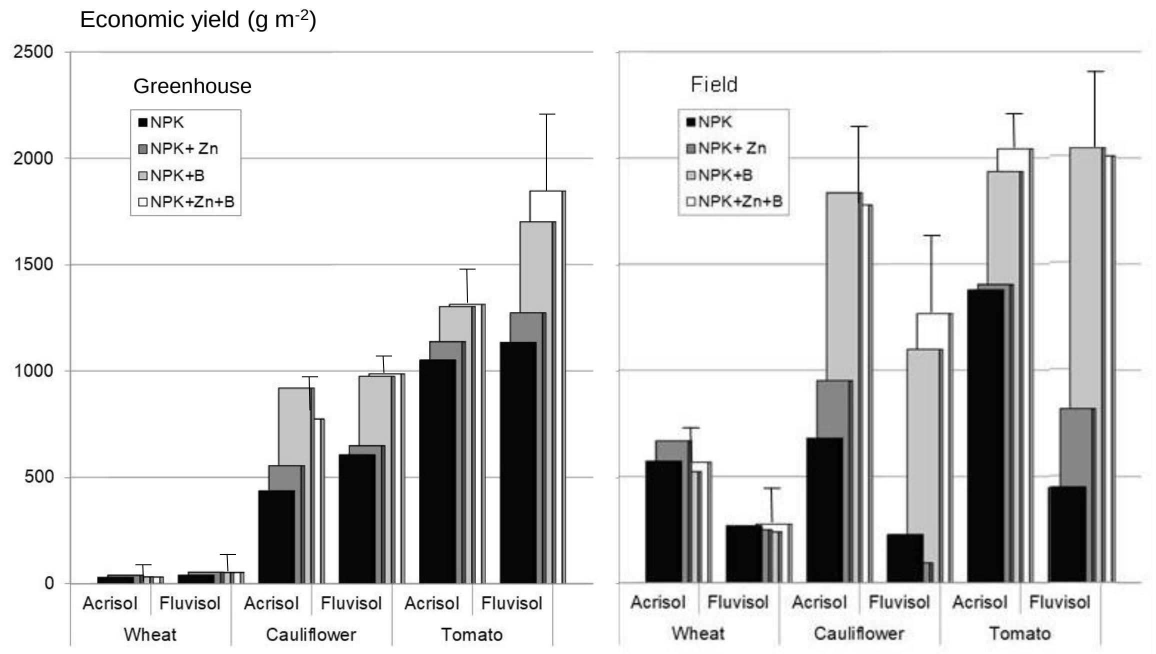
Site/Soil	System#	Treatments	Zn uptake (mg m^{-2}) in greenhouse			Zn uptake (mg m^{-2}) in the field		
			Wheat	Caulifl	Tomato	Wheat	Caulifl	Tomato
Mid-hills (Acrisols)	Rice	NPK	3.31 ns	4.81 ns	5.95 ns	10.20 b	2.81 b	1.60 b
		NPK+Zn	5.43	6.11	7.25	21.34 a	9.26 b	2.42 b
		NPK+B	2.78	4.14	6.46	10.26 b	30.98 a	3.70 ab
		NPK+Zn+B	4.13	5.14	6.65	16.63 ab	41.12 a	5.46 a
	Maize	NPK	1.80 ns	5.00 ns	8.17 bc	23.68 b	25.03 ns	3.19 ns
		NPK+Zn	1.98	7.23	11.55 a	37.33 a	36.00	4.90
		NPK+B	2.16	6.17	7.39 c	35.92 a	28.58	3.34
		NPK+Zn+B	1.92	8.88	9.69 ab	46.92 a	34.59	7.85
Lowland (Fluvisols)	Rice	NPK	0.99 b	3.62 ns	4.87 ns	1.51 c	19.74 ns	1.99 ns
		NPK+Zn	2.47 a	3.66	4.83	2.09 c	32.48	2.97
		NPK+B	1.61 ab	3.97	4.35	4.22 b	32.60	3.34
		NPK+Zn+B	2.71 a	4.50	4.38	7.15 a	35.16	3.45
	Maize	NPK	0.76 ns	1.65 c	1.59 b	8.40 ns	5.94 b	3.17 ns
		NPK+Zn	0.51	6.12 a	2.61 ab	11.73	25.23 b	6.15
		NPK+B	0.84	3.13 b	3.60 a	7.19	70.25 a	10.04
		NPK+Zn+B	0.89	4.17 b	3.32 a	8.83	76.54 a	11.06
Site / Soil			***	***	***	***	***	ns
System			***	*	ns	***	***	***
Site / Soil x system			ns	**	***	***	ns	ns
Treatment			*	***	***	***	***	***
Site / Soil x treatment			ns	ns	**	***	***	ns
System x treatment			*	*	ns	ns	ns	ns
Site x system x treatment			ns	ns	*	***	***	ns

Preceding crop during the wet season; x interaction between factors. Data values followed by the different letters within a column are significantly different by Least Significant Difference test (LSD) at p<0.05; ns = non-significant; * significant at p<0.05; ** significant at p<0.01 and *** significant at p<0.001. Zn concentration in the greenhouse: wheat: 19-61 mg Zn kg^{-1}; cauliflower: 43-147 mg Zn kg^{-1}; tomato: 46-79 mg Zn kg^{-1}. Zn concentration in the field: wheat: 8-28 mg Zn kg^{-1}; cauliflower 36-98 mg Zn kg^{-1}; tomato: 33-129 mg Zn kg^{-1}.

Table 4.3: Effect of mineral fertilizer application on boron uptake at early reproductive stage of wheat, cauliflower and tomato grown in the greenhouse (Acrisols and Fluvisols) in Kathmandu and under field conditions (mid-hills and lowland), in two production systems (rice vs. maize as preceding wet season crops), Nepal (2017).

Site/Soil	System#	Treatments	B uptake ($mg\ m^{-2}$) in the greenhouse			B uptake ($mg\ m^{-2}$) in the field		
			Wheat	Caulifl	Tomato	Wheat	Caulifl	Tomato
Mid-hills (Acrisols)	Rice	NPK	0.42 b	0.82 b	1.18 b	1.38 ns	0.34 c	0.39 b
		NPK+Zn	0.53 b	0.86 b	1.30 b	0.95	0.75 c	0.48 b
		NPK+B	2.65 a	3.99 a	4.41 a	2.59	46.72 a	2.57 a
		NPK+Zn+B	2.89 a	3.20 a	3.98 a	1.84	28.37 b	2.79 a
	Maize	NPK	0.23 b	0.78 b	1.56 b	1.48 b	3.59 b	0.91 b
		NPK+Zn	0.22 b	0.80 b	2.41 b	1.53 b	1.94 b	0.62 b
		NPK+B	0.93 a	5.20 a	6.07 a	4.95 a	25.59 a	1.74 ab
		NPK+Zn+B	0.92 a	5.62 a	5.45 a	5.07 a	22.66 a	2.55 a
Lowland (Fluvisols)	Rice	NPK	0.30 b	1.82 b	1.48 b	1.88 bc	9.09 c	1.15 b
		NPK+Zn	2.37 a	0.78 b	1.54 b	1.63 c	21.22 bc	2.34 a
		NPK+B	3.06 a	5.87 a	3.40 a	2.58 b	52.02 a	3.25 a
		NPK+Zn+B	2.58 a	5.10 a	3.13 a	4.13 a	33.87 b	3.03 a
	Maize	NPK	0.27 b	0.59 c	0.36 b	1.81 c	0.65 c	0.78 b
		NPK+Zn	0.10 b	1.05 c	0.55 b	0.75 c	0.81 c	0.84 b
		NPK+B	1.48 a	7.73 a	2.60 a	4.37 b	96.11 a	14.56 a
		NPK+Zn+B	1.28 a	6.43 b	2.59 a	10.30 a	82.19 b	10.99 ab
Site / soil			ns	***	***	***	***	***
System			***	**	ns	***	*	*
Site / soil x system			ns	ns	***	ns	***	**
Treatment			***	***	***	***	***	***
Site / soil x treatment			ns	**	***	***	***	**
System x treatment			***	***	**	***	***	**
Site / soil x system x treatment			ns	***	ns	*	***	**

Preceding crop during the wet season; x interaction between factors. Data values followed by the different letters within a column are significantly different by Least Significant Difference test (LSD) at p<0.05; ns = non-significant; * significant at p<0.05; ** significant at p<0.01 and *** significant at p<0.001. B concentration in greenhouse: wheat: 6-49 mg B kg^{-1}, cauliflower: 10-101 mg B kg^{-1} and tomato: 10-51 mg B kg^{-1}. B concentration in field: wheat: 1-26 mg B kg^{-1}, cauliflower: 4-102 mg B kg^{-1} and tomato: 14-83 mg B kg^{-1}.



Interactions	Wheat	Cauliflower	Tomato
Soil	***	**	**
System	***	**	***
Soil x system	ns	***	***
Treatment	ns	***	***
Soil x treatment	ns	ns	*
System x treatment	*	ns	**
Soil x system x treatment	ns	**	*

Interactions	Wheat	Cauliflower	Tomato
Site	***	***	ns
System	***	***	***
Site x system	***	***	***
Treatment	*	***	***
Site x treatment	*	ns	*
System x treatment	ns	***	*
Site x system x treatment	ns	**	***

Figure 4.2: Economic yield of wheat, cauliflower (fresh curd), and tomato (fresh fruits) grown in the greenhouse in two potted soils (Acrisols vs. Fluvisols) and under field conditions at two sites. Different patterns indicate the economic yield gain due to the respective fertilizer treatment. Data present mean values from two production systems (rice vs. maize as preceding wet season crops) and 4 replications. Bars represent standard errors of the mean for the maximum economic yield in the treatment with NPK+B+Zn fertilizer application (n=8).

The economic benefits of Zn application were more modest, ranging from 74 (tomato) over 111 (wheat) to 94 € ha^{-1} (cauliflower). On average, partial factor productivities of applied fertilizers were low to moderate in wheat, with 1 € (no benefit) in the case of B but up to 8 € increase in net revenue per € of investment in Zn fertilizer. In the vegetables, this partial factor productivity was approximately 4 € €$^{-1}$ investment with Zn fertilizer, and up to 43 € €$^{-1}$ investment in the case of B application, irrespective of the production site or cropping system (Table 4.4).

Table 4.4: Partial budget analysis with application of B and Zn on the wheat, cauliflower (fresh curd) under field conditions at two sites (mid-hills vs. lowland) and two production systems (rice vs. maize as preceding wet season crops), Nepal (2017).

Parameters	Wheat	Cauliflower	Tomato
Cost of B fertilizer - Borax (€ kg^{-1})	0.99	0.99	0.99
Cost of Zn fertilizer – Zn-Sulfate (€ kg^{-1})	0.94	0.94	0.94
Borax application rate (kg ha^{-1})	18	40	20
Zn-Sulfate application rate (kg ha^{-1})	15	20	20
Cost of B application (€ ha^{-1})	18	40	20
Cost of Zn application (€ ha^{-1})	14	19	19
Price of commodity (€ t^{-1})	192	154	192
Yield gain due to B application (t ha^{-1})	0.10	11.13	10.08
Yield gain due to Zn application (t ha^{-1})	0.58	0.59	0.25
Monetary gain due to B application (€ ha^{-1})	19	1,714	871
Monetary gain due to Zn application (€ ha^{-1})	111	94	74
Partial factor productivity of B (€ gain €$^{-1}$ investment)	1.10	42.85	43.54
Partial factor productivity of Zn (€ gain €$^{-1}$ investment)	7.90	4.90	3.80

Boron application as borax at 4.4, 2.2, and 2.0 kg B ha^{-1} in cauliflower, tomato, and wheat, respectively.

Zinc application as Zn-sulfate at 4.4 kg Zn ha^{-1} in cauliflower and tomato and 3.3 kg Zn ha^{-1}in wheat.

4.4 Discussion

The following sections discuss (1) soil attribute changes due to system shifts from rice to maize, (2) the role of B and Zn applications on crop performance attributes and the economic productivity and (3) the economic benefits (here partial budget analysis) and the likely implications of the findings regarding recommendations for Nepal and further research needs.

4.4.1 System shifts from rice to maize reduce soil fertility and alter Zn availability

The soils at the two study sites are representative of the prevailing edaphic conditions in the mid-hills (acid Acrisols) and subtropical lowland (sandy Fluvisols) of Nepal. Thus, acid Acrisols with sandy clay loam texture dominate the mid-hills areas of central and eastern Nepal, while sandy loam Fluvisols prevail in much of the lowland in the Terai, the lowland areas in the IGP region of Nepal (Andersen 2007). Most soils in the region are sandy soils with low to very low B and Zn contents (Padbhushan and Kumar 2017). Coarse-textured materials dominate the Himalayan mid-hills in both India (Shukla et al. 2017) and in Nepal, and are also typically associated with sandy Fluvisols in the IGP of Nepal (Kataki et al. 2001). High rainfall intensities during the monsoon season further deplete soil nutrient resources by erosion and leaching (Acharya et al. 2005). This is particularly critical in the acid Acrisols of the mid-hills where B is present as easily leachable boric acid (Goldbach 2020). Both B and Zn are further easily removed by leaching with high rainfall intensities in the highly percolating sandy soils (Shaaban 2010). As a consequence, majority of rice-growing area of Nepal is currently characterized by Zn-deficient soils (Andersen 2007), with 2-3 mg Zn kg^{-1} of available (DTPA-extractable) Zn, being just at the limit of the sufficiency range for maize and rice (0.8 – 3.4 mg Zn kg^{-1}). The water or $CaCl_2$-extractable soil B of <0.3 mg kg^{-1} (Ahmed and Hossain 1997) is below the critical limit for growing cereals (0.5 mg kg^{-1}) and for dicotyledonous vegetables (1.5 mg kg^{-1}) (Gupta 1968; Goldbach 2020).

A shift in wet season cropping from lowland rice to upland maize under *ceteri-paribus* conditions may cause several changes in soil attributes. While it only slightly reduced the soil pH value by 0.2-0.3 units, it significantly reduced soil total C and N contents in the present study (Table 2.2). As has been shown before, soil submergence can contribute to conserving soil C (Kirk 2004), while soil aeration can accelerate soil microbial mineralization processes (Lal 2015), thus reducing soil C and N contents (Gale and Gilmour 1988). In the present study, this decline in soil fertility was associated with a reduction of dry matter accumulation, especially of pot-grown wheat. In contrast to wheat, the vegetables appeared largely unaffected by reduced soil C and N contents, irrespective of the experimental conditions (greenhouse vs. field) or the site (mid-hills vs- lowland).

In addition, most micro-nutrient minerals (including Zn, but not B) are better plant available in their reduced state and hence a shift towards more aerobic crop production in formerly lowland rice-based systems will likely exacerbate conditions of low soil micronutrient content in Nepal (Gami et al. 2001). On the other hand, rice is a highly Zn-demanding crop (Dobermann and Fairhurst 2000), and the formation of ZnS or Zn sorption to Fe-hydroxides can also reduce its availability for plants. Thus, continued rice cultivation has probably contributed to the low available soil Zn content (Rehman et al. 2018) in both soils/sites. The shift to wet season maize has reduced the Zn removal by crop uptake and is reflected in slightly higher soil Zn contents compared to the rice-based systems. Therefore, replacing wet-season rice by maize may lead to a larger pool of Zn in the soil, which, however, might be less available. Whether this is beneficial or detrimental to the crop production will likely depend on climatic conditions and the Zn demand of chosen dry season crops. Both soils reflect the insufficient B supply commonly observed in Nepal. A system shift to wet-season maize did not consistently change the available soil B content, which was expected since B availability does not largely depend on the reduction state of the soil. In addition, aerobic conditions did not significantly reduce the soil pH values, and thus the system shift from rice to maize is not expected to lead to increased leaching events.

Overall, it can be concluded that the emerging production systems replacing rice by maize negatively affect soil C and N contents and may aggravate soil Zn deficiencies in Nepal, pointing towards a growing need to build soil C and N stocks and to externally apply the missing Zn.

4.4.2 Crop diversification leads to increased fertilizer demand

Micronutrient deficiencies are considered the principal cause for declining productivity in several cultivation systems in the IGPs (Mondal et al. 2015), and low soil B and Zn contents are recurrently acknowledged as being in part responsible for the reported productivity declines of rice-wheat rotation systems in Nepal (Gami et al. 2001; Akhtar et al. 2013). Despite only small amounts being required, these two micronutrients are essential for crop growth, and the demand, especially for B, is overall higher in horticultural crops than in cereals (Broadley et al. 2012).

The reduced soil availability and increased crop demands for B and Zn with ongoing system shifts have been demonstrated for both lowland and mid-hills sites in

Nepal (Chapter 3), pointing to the growing need of applying B and Zn fertilizers in the emerging vegetable-based systems. In the present study, focus on the different site- and crop-specific response patterns to B and Zn fertilizer applications, and its implications for economic benefits.

Boron effects: Boron is essential for both growth and development, and species-specific threshold concentrations in plant tissues must be attained before the element is to fully achieve its functions. In most well-fed crops, B concentrations in plant tissues range between 10 and 100 mg B kg^{-1}, while >100 mg B kg^{-1} is a common threshold for B toxicity (Gupta 2007). However, species demands for B differ significantly, and minimum tissue B concentrations are generally lower in cereals than in dicotyledonous plants (Wimmer et al. 2015). For example, the critical B thresholds for the dry season crops used in the present study are 10-15 mg B kg^{-1} in wheat (Hu et al. 1996), 30-75 mg B kg^{-1} for tomato and 12-97 mg B kg^{-1} for cauliflower (Wimmer et al. 2015).

The most relevant B deficiency symptoms affecting yield and market value are high rates of spikelet sterility in wheat, hollow stems, the formation of brown necrotic spots on the curd, and rotting of the core in cauliflower, and injuries of the growing point and flower casing, leading to reduced fruit-setting or corky fruits in tomato (Goldbach 2020).

In Nepal, the widespread occurrence of B deficiency was reported to cause sterility in wheat (Subedi 1992), and the application of 1 mg B kg^{-1} soil increased tissue B concentrations more than 25% in the leaves of field-grown wheat in Bangladesh (Saifuzzaman 1995) and significantly improved several growth parameters and the grain yield of rice as well as of the succeeding pot-grown crop of wheat in both red mid-hill soils as well as in alluvial lowland soils of India (Kumar and Singh 2018) and approximately 14% yield increased in Pakistan (Rashid et al. 2002). Applying 2 kg B in the form of borax at seeding also increased the grain yield of field-grown wheat (Singh 2008; Nadeem and Farooq 2019). In the present study, application of 2 kg B ha^{-1} increased biomass accumulation and B uptake by wheat, but yield effects were not significantly affected in either greenhouse or field, and irrespective of the site or the system. Given that B tissue levels in wheat leaves were below the critical level reported for wheat in both pot and field trials, this result might indicate that other factors besides low B availability limited biomass production of wheat in this study.

For vegetables, the responsiveness to B fertilization regarding biomass, B uptake and economic yield was more pronounced than for wheat in both pot and field conditions. This is not surprising since dicotyledonous plants are usually more sensitive to B deficiency and require larger amounts of applied B (Shaaban 2010). In previous studies, benefits of B application for tomato quantity and quality were observed in the Himalayan mid-hills of India on acid soils (Verma et al. 2018), as well as in Bangladesh (Salam et al. 2010). In the present study, application rates of 2.0 to 4.4 kg B ha^{-1} raised the tissue B levels clearly above the critical values, and corresponding yield increases were economically highly profitable.

The plants of the Brassicaceae family have a relatively high demand for B (Singh 2008).This is reflected in the pronounced positive yield response of cauliflower to B fertilizer application, which is in line with reports of cauliflower yield increases of up to 100% in B deficient regions of China (Zou et al. 2008), more than 50% in the mid-hills (Khadka et al. 2005) and the lowland regions (Dhakal et al. 2014) of Nepal and more than in India and in Bangladesh (Ahmed and Hossain 1997; Batabyal et al. 2015; Hassan et al. 2018; Padbhushan et al. 2019).

Interestingly, the total B uptake into the biomass of all field-grown dry season crops was higher at the lowland site than at the mid-hills site, while no differences were observed in the greenhouse. It is a typical feature of B deficiency that symptoms are related to the plant's internal B translocation, since B transport is mainly driven by the transpiration stream (Brown et al. 2002). Thus, the relative humidity is a key environmental factor affecting B translocation. Under conditions of high vapor pressure deficit (VPD), i.e. during the dry season at the lowland site, transpiration thus likely enhanced B translocation to transpiring organs (Hu and Brown 1997), leading to a corresponding better growth and yield especially in cauliflower and tomato. The lack of such an effect in the pot trial is fully in line with the similar VPD for plants grown in either Fluvisols or Acrisols. This transpiration dependency also underpins the large sensitivity of cauliflower and tomato to conditions of soil B deficiency, with the harvested organs (curd and fruits) being weakly-transpiring, and consequently showing low B translocation to the site of need. In addition, B can be immobilized in stems and petiole tissues of tomato, a fact that further exacerbates the insufficient B translocation to the reproductive organs, and leads to poor tomato quantity and quality (Choi et al. 2015). And the application of B fertilizers increased the growth, yield and nutrient content of tomato (Davis et al. 2003).

Even though the economic yield of vegetables grown at the lowland site without micronutrient addition (NPK treatment) was lower than at the mid-hills site, this difference was minimized by B application and resulted in reasonable economic yields for both cauliflower and tomato (see Appendix 3,4). The B uptake per gram of biomass production was similar between both sites (data not shown), indicating that the additional B was directly used for biomass production.

Zinc effects: Zinc is involved in enzymatic reactions, protein synthesis and carbohydrate metabolism, affecting photosynthesis and sugar transformations in plants (Barker and Eaton 2015). It is involved in regulating the synthesis and oxidative degradation of the growth hormone auxin and under deficiency conditions, roots exude large amounts of amino acids and phenolic compounds (Cakmak 2000). In the present study, the typical Zn deficiency symptoms such as interveinal chlorosis of leaves and stunted growth have been widely apparent in young wheat plants grown under field conditions, particularly in the Fluvisols at the lowland site. They correlated with Zn tissue concentrations of non-amended wheat close to maturity which were below the critical threshold level of 15-20 mg kg^{-1} for wheat (Alloway 2008) in both soils. On the other hand, for vegetables, tissue Zn concentrations overall exceeded the critical limits of 20 mg kg^{-1} for cauliflower and 25 mg kg^{-1} for tomato (Hochmuth et al. 2015).

The addition of Zn fertilizer increased tissue Zn concentrations and the dry matter accumulation of both wheat and cauliflower especially in the field but to different extents. Despite significant Zn-induced increases in Zn concentrations and Zn uptake in wheat, irrespective of the site/soil, these effects only slightly improved economic yield. This could be explained by the fact that the Zn application did not suffice to raise the Zn tissue levels above the critical limit under field conditions, and overall growth of wheat was likely limited by Zn supply even in the fertilized treatments. On the other hand, Zn application to vegetables also increased Zn uptake and biomass yield under both pot and field conditions, but since tissue levels were above the critical values even without Zn application, the effect of Zn supply alone on biomass yield was not very pronounced, compared to the beneficial effect of additional B supply. The benefits of Zn application were observed of tomato grown in Zn deficient soils (Kaya and Higgs 2002), also reported in Bangladesh (Salam et al. 2010).

Collectively, the results of this study indicate that in the B and Zn deficient soils of Nepal, B and Zn applications are required to stimulate plant growth, nutrient uptake and economic yield. However, the required type of fertilizer and the crop- and site-specific responsiveness differ, with Zn application being more relevant for wheat-based systems (especially in rice-based systems of the mid-hills), while additional B supply is more important for vegetable-based systems.

4.4.3 Micronutrient fertilization provides economic benefits with system shifts

The distinct growth responses of crops to micronutrient supply are also reflected in economic benefits for farmers. Monetary gain of investing in Zn fertilizer was significant in wheat (up to 8 € $€^{-1}$ investment), but only marginal in vegetables (up to 4 € $€^{-1}$), while partial factor productivity of > 40 € $€^{-1}$ investment suggests borax use to be highly profitable in vegetables, irrespective of the production site or cropping system. Economic benefits similar to those reported here have been shown for Zn application to wheat in Turkey (Cakmak 2008a) and B application to cotton and maize in Pakistan (Rashid et al. 2007). Overall, the need for applying B and / or Zn fertilizers differs between production systems, sites, and soils, but is expected to increase with progressing system shifts. At present, the applications of Zn fertilizer can improve the performance of the conventional rice-wheat rotation in the relatively cool mid-hills environment. With progressing urbanization and the concomitant shift towards high-value vegetable-based systems, the crops' demand for B will increase, and the external application of B-containing fertilizers will become essential. However, farmers in Nepal are rarely aware of the problem and most cannot relate the wide-spread visual symptoms to Zn and B deficiencies. In addition, neither Zn nor B fertilizers are presently available in the rural area of Nepal. Without the recognition of the B and Zn deficiency problematics and without the possibility to correct the deficiencies through amendments, the ongoing shift in production systems will in the longer-term neither meet the changing demand of urban consumers nor the financial expectations of farmers, including their returns to investments in agricultural innovations.

4.5 Implications and recommendations

Continuous changes in cropping practices and the adoption of technology innovations are cyclic phenomena of adaptation to changing conditions that were first conceptualized in the “adaptive cycles theory” by the Canadian ecologist Buzz Holling (Allen et al. 2014). Thus, external pressures and system-immanent drivers result in resource mismatches and necessitate adaptive change in production systems. Failure or incapacity to adapt to change can push systems to the “edge of collapse” and a decoupling of the social-ecological system. The speed and extent to which farmers are able to respond to pressures and drivers by modifying their agro-production system and by adopting new practices that help counteracting resource mismatches is a dimension of system resilience (Becker and Angulo 2019). While being first mentioned as an agricultural practice in the Song Dynasty between 960 and 1200 B.C (Xiongsheng 2005), the rice-wheat annual double cropping system emerged on large scale in the 1970s as an adaptation of intensive irrigated green revolution technologies to the sub-tropical conditions in the IGP and the Himalayan mid-hills (Harrington and Hobbs 2009). Its wide-spread adoption, initially in India and later in Bangladesh, Pakistan and Nepal, was able to largely meet the growing food needs associated with the large demographic growth rates in much of South Asia and generate additional income for farmers (Byerlee 1992). The newly emerging systems in Nepal that are addressed in this study reflect an adaptation to changing conditions in the social (i.e. urbanization and changing consumer demands) and in the ecological sub-systems (i.e. emerging water shortages), attesting to the adaptive capacity of Nepalese farmers. This adaptive change will however be only partial and is unlikely to meet societal demand and farmer expectations if the changing /increasing demand for the critically limiting micronutrient elements Zn and B cannot be met. To prevent the failure of the emerging systems and avoid the social-ecological agro-production systems of Nepal, several measures need to be undertaken both within the public and the private sectors:

(1) The Nepal Agricultural Research Council (NARC) as the leading national research institution must support efforts to develop and refine environment-, soil-, system-, and crop-specific B and Zn fertilizer recommendations. Department of Agriculture, Agriculture Information and Training Centre should prepare comprehensible extension aid materials for farmers according to crops and systems.

(2) The Government of Nepal, Department of Agriculture, as well as other national and international extension services providers must ensure awareness creation among farmers about the need for B and Zn, particularly in the emerging production systems.

(3) The private sector, international fertilizer agencies, and traders must recognize that with the on-going system shifts, Nepal is no longer a niche market, but that the development of new formulations of B and Zn fertilizers, their site- and crop-specific packaging, and their provision within local markets is economically profitable for farmers and for supplies.

Chapter 5

Based on the journal article:

Shrestha S, Becker M, Lamers JPA, Wimmer MA (2020c) Residual effects of B and Zn fertilizers applied to dry season crops on the performance of the follow-up crop of maize in Nepal. Journal of Plant Nutrition and Soil Science. (Paper accepted, jpln.202000289)

5 RESIDUAL EFFECTS OF B AND ZN FERTILIZER APPLIED TO DRY SEASON CROPS ON THE PERFORMANCE OF FOLLOW-UP CROP MAIZE IN NEPAL

Abstract

Diversified food crop production systems with high-value vegetable crops are increasingly replacing conventional rice-wheat rotations across Nepal. These emerging systems enhance the demand for boron (B) and zinc (Zn), which are already widely deficient in Nepal. However, B and Zn fertilizers are often either unavailable in rural areas, not available in a timely manner, or when available not affordable by smallholder farmers. To overcome these multiple constraints, the current study assessed whether soil stock provisioning, e.g. B and Zn fertilizer applications to dry season crops, may provide sufficient residual effects for the succeeding non-fertilized wet season crop. A pot experiment was conducted in a sheltered greenhouse using two contrasting soil types (Acrisols and Fluvisols) collected from rice- and from maize-based production systems in Nepal. B and Zn were supplied individually or in combination to wheat, cauliflower and tomato grown in the dry season, and biomass accumulation, B and Zn concentrations as well as B and Zn uptake were determined in the unfertilized follow-up crop of maize. Soil-applied B and Zn fertilizers, sole and combined, only slightly enhanced biomass of the succeeding non-fertilized maize, but significantly increased B and Zn uptake, with significant differences between soil types. While maize shoot B concentrations were consistently increased above the critical levels in both soils, but especially in the Fluvisols after wheat and cauliflower pre-crops, Zn uptake of maize was more enhanced in the Acrisols. The residual effects of B and/or Zn applied to preceding crops benefitted the subsequent wet season maize, at least at certain sites and after certain pre-crops, and may suffice to attain reasonable yields of wet season crops in the emerging vegetable-based cropping systems of Nepal, even if fertilizers are not consistently available. The magnitude of the residual effects of pre-applied Zn largely depended on the soil type, while those of B were mainly determined by the pre-crop.

5.1 Introduction

Rice-wheat rotations currently are the predominating agricultural production system in the Indo-Gangetic Plains (IGP) and the Himalayan mid-hills of India (Chauhan et al., 2012) as well as in Nepal (Regmi et al., 2002). However, the conventional rice-wheat rotation is currently subject to changes mainly because farmers are replacing wet season rice by the less water-demanding crop of maize, or in other regions by replacing the low-value dry season wheat by high-value vegetables such as tomato or cauliflower. Yet, such agricultural intensification and crop diversification practices may negatively affect soil fertility (Ladha et al., 2003) and specifically exacerbate the already widely-occurring soil deficiencies of key micronutrients such as zinc (Zn) and boron (B) (Nadeem and Farooq, 2019), as was recently also shown for Nepal (Shrestha et al., 2020a). Even though only small quantities of micronutrients are required, Zn deficiency is currently limiting the growth of lowland rice on an estimated 50% area of the global production (Cakmak, 2008), but particularly in India, Pakistan and Nepal (Alloway, 2008). Similarly, B deficiency is a major constraint to many crops (Shorrocks, 1997) in at least 80 countries worldwide (Sillanpää, 1982), but it is particularly cumbersome in Nepal (Rerkasem and Jamjod, 2004; Shorrocks, 1997). Hence, matching the in-season demands for these micronutrients with targeted supplies of B and Zn is likely to enhance both crop production and product quality as has been shown recently for the emerging vegetable-based rotation systems of Nepal (Shrestha et al., 2020b).

However, regular applications, particularly of B-fertilizers, also can easily induce toxic soil levels (Gupta et al., 1985) with subsequent negative effects on crop performance (Singh and Abrol, 1985). In addition, regular applications of B and Zn fertilizers in Nepal are currently also constrained by their limited availability in rural areas, due to an often-untimely availability of fertilizers on the markets, and if available by their non-affordability for smallholder farmers (Shrestha et al., 2016). It would thus be advantageous not having to apply these fertilizers to every crop or in every year, and to rely on residual or carry-over effects of applied B and Zn on follow-up crops.

Such multi-year effects of one-time applications also depend on the fact that most crops use only a fraction of the applied micronutrients (Eguchi and Yamada, 1997a., Yang et al., 2000). Hence, non-absorbed nutrients can benefit subsequent crops, whereby the magnitude of such residual effects depends on the amount and mode of the initial fertilizer application (Shorrocks, 1997), the crops grown, as well as on soil properties (Martens and Westermann, 1991). For instance, residual benefits of a one-time B fertilizer application benefitted subsequent crops of rice or oilseed rape more in Inceptisols than in Ultisols (Rehman et al., 2018; Yang et al., 2000), and a long-term positive effect on wheat yields was observed in rice-wheat rotations in Pakistan (Rashid et al., 2007). Similarly, increased Zn concentrations and higher yields of a non-amended crop of maize were observed following Zn applications to a rice field on a lowland calcareous soil in China (Liu et al., 2017).

It appears thus reasonable to assume that, despite their low application rates, B and Zn fertilizers applied to different dry season crops may benefit non-fertilized subsequent maize, but the extent of such an effect may likely depend on the soil type and the production system. Therefore, we investigated the residual effects of B and Zn fertilizer applications to wheat, tomato and cauliflower on maize grown in potted soils of different origins (Fluvisols from the lowland and an Acrisols from the mid-hills) and from representative production systems in Nepal (e.g. rice-based /anaerobic vs. maize-based / aerobic soil conditions during the wet season; for details see Shrestha et al., 2020a). Carry-over effects were assessed by comparing biomass accumulation, tissue concentrations and total uptake of B and Zn.

5.2 Material and methods

5.2.1 Study site

The residual experiment was conducted as a pot trial in a greenhouse of the Nepal Agricultural Research Council (NARC) in Kathmandu. The potted soils were collected from two field sites originating from areas with distinct edaphic and climatic conditions, representing the mid-hills (Acrisols, Kavre site) and the lowland (Fluvisols, Rampur site) of Nepal. At both sites, soils were collected from rice-wheat and maize-wheat rotations. The collected soils were translocated to the study site in Kathmandu and analyzed for their physical-chemical attributes (the details of soil of analysis see section 2.3). The $CaCl_2$ extractable soil B content ranged from 0.14 to 0.30 in both soils and was thus critical (below 0.5 mg B kg^{-1}: Sah and Brown, 1997). The soil available Zn content was on average 2.1 mg kg^{-1} for the Fluvisols and 2.7 mg kg^{-1} for the Acrisols, which was close to or slightly above the critical limit for rice growth (Dobermann and Fairhurst, 2000). Inside the rain-sheltered greenhouse, the mean temperature during the study period was maintained at 20-25°C with an air humidity of approximately 70%. For further details of climatic conditions see Shrestha et al. (2020a).

5.2.2 Experiment design and treatments

Approximately 8.8 kg dried and sieved topsoil (0 - 20 cm) collected from both sites and production systems were filled into 10-L plastic pots. These experimental pots were cultivated during the 2018 dry season with either wheat with vegetables (cauliflower and tomato) and either received only the recommended mineral NPK fertilizers, or NPK combined with the crop-specific recommended rates of 2.0 kg B ha^{-1} (wheat), 2.2 kg B ha^{-1} (tomato) and 4.4 kg B ha^{-1} (cauliflower), and of 3.3 kg Zn ha^{-1} (wheat), and 4.4 kg Zn ha^{-1} (cauliflower and tomato), either singly or in combinations. In total, 48 treatment combinations, comprising 2 soil types x 2 systems x 3 dry season crops x 4 fertilizer treatments, were arranged in a completely randomized design using 4 replications (in total 192 pots). Further details regarding treatment applications are reported in Shrestha et al. (2020b).

5.2.3 Crop management and analyses

After harvesting the dry season crops in the pots and the removal of all aboveground biomass, the top 5 cm of each pot were manually tilled and a uniform crop of non-fertilized maize (namely the hybrid genotype Manakamana-4, which is tolerant to *Turcicum* leaf blight and is recommended by the Agriculture botany division, NARC) was established by adding 5 seeds per pot. Soil moisture during the entire experiment was maintained at about 70% field capacity by weekly weight-adjusted irrigation with demineralized water. The aboveground biomass of approximately 5-week-old maize plants was harvested in June 2018. The biomass was oven-dried at 60˚C for 72 hours to constant weight, weighed and ground in a sample mill (FT102 Micro Soil Disintegrator) to pass a 0.5 mm sieve. Sub-samples of this dry biomass were analyzed for Zn (atomic absorption spectrometry) and B (spectrophotometrically by a miniaturized curcumin method: Wimmer and Goldbach 1999) at the Institute of Crop Science and Resource Conservation of the University of Bonn, Germany. Total B and Zn uptake was calculated by multiplying the B and Zn contents (mg kg^{-1}) of the dry biomass with the respective dry weight (mg pot^{-1}) of the plants. Details to all analytical procedures have been reported in Chapter 2 section 2.8).

5.2.4 Statistical analysis

Data was analyzed using one-way analysis of variance in STAR (version 2.01) and mean difference were separated using LSD test at p = 0.05. The interaction between site, system, and preceding crop analyzed using mix model in Stata 14.2. Mean is comparing by pairwise comparison between and within factors. B and Zn concentrations and uptake were presented graphically using a bar chart in excel and their performance variability was assessed using standard errors of means.

5.3 Results

5.3.1 Dry matter accumulation

The dry aboveground biomass of non-fertilized maize overall ranged from 4.1 to 8.3 g pot^{-1}, depending on the soil type, system, treatment and preceding crop (Table 5.1). It differentially responded to all factors, but particularly to residual B and Zn fertilizers applied to the pre-crops (Table 5.1). Residual effects on maize biomass were not significant with the exception of Zn and B applications to a pre-crop of tomato in the rice-based Fluvisols, as well as Zn application to a pre-crop of wheat in the rice-based Fluvisols and of tomato in the maize-based Fluvisols (Table 5.1). Even though maize dry matter responded relatively little to residual micronutrients, it was at least slightly enhanced as compared to NPK alone in 30 out of 36 treatment combinations, irrespective of the soil type or the soil aeration status.

Table 5.1: Residual effect of Zn and B fertilizers applied to preceding dry season crops of wheat, cauliflower or tomato on the dry matter accumulation of a non-amended maize, grown in potted soils of different origin (Fluvisols from a lowland and Acrisols from a mid-hill site) and from different production systems (rice vs. maize as preceding wet season crops) (greenhouse experiment in Kathmandu, Nepal in 2018).

Soil	System#	Treatments	Dry matter accumulation (g pot^{-1}) by maize								
			Preceding dry season crops								
			Wheat			Cauliflower			Tomato		
Acrisols	Rice	NPK	4.47	±0.26	ns	4.74	±0.09	ns	5.71	±0.46	ns
		NPK+Zn	4.69	±0.16		5.73	±0.31		7.49	±1.6	
		NPK+B	5.39	±0.43		5.66	±0.55		6.07	±0.57	
		NPK+Zn+B	4.84	±0.31		6.53	±1.14		6.03	±0.53	
	Maize	NPK	5.71	±0.46	ns	4.15	±0.33	ns	4.14	±0.19	ns
		NPK+Zn	7.49	±1.60		4.41	±0.41		4.72	±0.16	
		NPK+B	6.07	±0.57		4.37	±0.54		5.39	±0.43	
		NPK+Zn+B	6.03	±0.53		3.95	±0.17		4.84	±0.31	
Fluvisols	Rice	NPK	6.91	±0.06	ab	5.29	±0.17	ns	4.49	±0.23	b
		NPK+Zn	8.32	±1.12	a	5.87	±0.23		5.70	±0.46	a
		NPK+B	6.54	±0.65	ab	5.73	±0.50		5.82	±0.18	a
		NPK+Zn+B	5.64	±0.61	bc	4.63	±0.11		5.68	±0.34	a
	Maize	NPK	5.14	±0.45	ns	6.45	±0.37	a	6.37	±0.56	ab
		NPK+Zn	5.70	±0.46		7.01	±0.24	a	8.32	±1.12	a
		NPK+B	5.82	±0.18		5.11	±0.36	b	6.14	±0.70	b
		NPK+Zn+B	5.68	±0.34		6.35	±0.22	a	6.17	±0.29	b
Site/Soil			ns			***			ns		
System			ns			ns			ns		
Site/Soil x system			***			***			***		
Treatment			ns			*			ns		
Site/Soil x treatment			ns			ns			ns		
System x treatment			ns			ns			ns		
Site/Soil x system x treatment			ns			ns			ns		

Preceding crop during the wet season with contrasting soil aeration status: maize aerobic, rice anaerobic; x interaction between factors. Data values followed by the different letters within a column are significantly different by Least Significant Difference test (LSD) at $p<0.05$; ns = non-significant; * significant at $p<0.05$; ** significant at $p<0.01$ and *** significant at $p<0.00$, ± standard error

5.3.2 Zinc and Boron concentrations

Soil-applied B and Zn fertilizers, sole and combined, consistently induced beneficial residual effects on the whole plant tissue nutrient concentrations of the succeeding maize, even though the magnitude of these effects differed depending on the soil type, the preceding crop as well as the micronutrient (Table 5.2, 5.3, Figure 5.1). When Zn fertilizer was applied to the dry season crops grown in the Acrisols, Zn contents in non-fertilized maize leaves increased on average by 24% e.g. from 43.5 ± 6.8 mg Zn kg^{-1} (without Zn amendments) to 53.8 ± 8.2 mg Zn kg^{-1} (with a previous addition of Zn) (average across all pre-crops and systems). The extent of this effect was most pronounced after tomato (+34%), followed by wheat (+28%) and cauliflower (+10%). However, the residual Zn effect was very little in the Fluvisols, where maize tissue Zn concentrations increased on average only by 11% e.g. from 22.3 ± 3.3 mg Zn kg^{-1} (without Zn amendments) to 24.8 ± 8.6 mg Zn kg^{-1} (with a previous addition of Zn) (Table 5.2). Again, the effect was strongest after tomato (+14%) followed by wheat and cauliflower (+9%). Despite this slight increase, and different from the Acrisols, Zn concentrations were never raised above the critical level of 32 mg Zn kg^{-1} (Rashid and Rafique, 1989) in the Fluvisols. The residual response to applied B was overall more pronounced especially in the Fluvisols (Table 5.2, 5.3). Maize leaf B concentrations increased on average by 190% in the Acrisols from initially 8.5 ± 3.1 mg B kg^{-1} without B applications to 24.7 ± 8.8 mg B kg^{-1} with a previous B fertilization. Here, the strongest effect was observed after tomato (+297%) and cauliflower (+289%), followed by wheat (+70%). In the Fluvisols, B concentrations raised on average by 581% from 7.6 ± 2.5 mg B kg^{-1} to 51.7 ± 23.7 mg B kg^{-1}. Strongest effects were observed after cauliflower (+878%), followed by wheat (+600%) and tomato (+247%). B application always increased maize leaf B contents above the critical threshold value of 9 mg B kg^{-1} (Gupta, 2007) (Figure 5.1) . In general, the residual response of leaf concentrations to B and Zn applications was more pronounced than that of the dry matter, and effects were higher for B than for Zn.

Table 5.2: Residual effects of Zn and B fertilizers applied to preceding dry season crops of wheat, cauliflower or tomato on the Zn and B leaf tissue concentrations of non-amended maize grown in potted soils of different origin (Fluvisols from a lowland and Acrisols from a mid-hill site; greenhouse experiment in Kathmandu, Nepal in 2018). Values are given as means of treatments without micronutrient addition ('-Zn' and '-B') and those with micronutrient addition ('+Zn' and '+B') ± standard deviations (n = 4). The lower row indicates mean values across all three pre-crops. Critical values for maize are 32 mg Zn kg^{-1} (Rashid and Rafique, 1989) and 9 mg B kg^{-1} (Gupta, 2007).

Zn tissue concentration of maize shoots (mg kg^{-1} DM)				
Pre-crop/Soil	Acrisols		Fluvisols	
	-Zn	+Zn	- Zn	+Zn
Wheat	40.3 ± 7.6	51.5 ± 5.2	24.5 ± 3.9	26.8 ± 2.1
Cauliflower	47.0 ± 3.7	51.8 ± 5.4	19.8 ± 1.9	21.5 ± 3.1
Tomato	43.3 ± 8.1	58.0 ±12.4	22.8 ± 2.2	26.0 ± 2.6
Mean	*43.5 ± 6.8*	*53.8 ± 8.2*	*22.3 ± 3.3*	*24.8 ± 8.6*

B tissue concentration of maize shoots (mg kg^{-1} DM)				
Pre-crop/Soil	Acrisols		Fluvisols	
	- B	+B	- B	+B
Wheat	11.8 ± 3.6	20.0 ± 3.6	7.3 ± 2.2	50.8 ± 6.7
Cauliflower	6.5 ± 1.0	25.3 ±13.1	8.0 ± 2.2	78.3 ±12.1
Tomato	7.3 ± 0.5	28.8 ± 4.0	7.5 ± 3.7	26.0 ± 8.5
Mean	*8.5 ± 3.1*	*24.7 ± 8.8*	*7.6 ± 2.5*	*51.7 ±23,7*

Preceding crop during the wet season

Table 5.3 : Residual effect of Zn and B fertilizers applied to preceding dry season crops of wheat, cauliflower or tomato on the Zn and B concentrations of non-amended maize, grown in potted soil of different origin (Fluvisols from a lowland and Acrisols from a mid-hill site) and from different production systems (rice vs. maize as preceding wet season crops) (greenhouse experiment in Kathmandu, Nepal in 2018).Values are given as means of treatments without micronutrient addition (‘-Zn’ and ‘-B’) and those with micronutrient addition (‘+Zn’ and ‘+B’) ± standard deviations (n = 4).

			Zn tissue concentration (mg kg^{-1} DM)					
Soil	**System#**	**Treatments**	Wheat	sd	Cauliflower	sd	Tomato	sd
Acrisosl	Rice	-Zn	37.0	±11.3	44.0	±1.4	49.5	±4.9
		+Zn	55.0	±5.7	49.0	±5.7	68.0	±5.7
	Maize	-Zn	43.5	±2.1	50.0	±1.4	37.0	±4.2
		+Zn	48.0	±0.0	54.5	±4.9	48.0	±5.7
Fluvisols	Rice	- Zn	26.0	±4.2	20.5	±0.7	21.5	±0.7
		+Zn	28.0	±1.4	24.0	±1.4	24.0	±1.4
	Maize	-Zn	23.0	±4.2	19.0	±2.8	24.0	±2.8
		+Zn	25.5	±2.1	19.0	±1.4	28.0	±1.4
			B tissue concentration (mg kg^{-1} DM)					
Acrisols	Rice	-B	9.0	±2.8	6.0	±1.4	7.0	±0.0
		+B	20.0	±11.3	35.5	±9.2	32.0	±1.4
	Maize	- B	14.5	±0.7	7.0	±0.0	7.5	±0.7
		+B	20.0	±2.8	15.0	±2.8	25.5	±2.1
Fluvisols	Rice	- B	8.5	±0.7	9.5	±2.1	10.5	±2.1
		+B	50.0	±8.5	71.5	±7.8	31.0	±9.9
	Maize	-B	6.0	±2.8	6.5	±0.7	4.5	±0.7
		+B	51.5	±2.1	85.0	±14.1	21.0	±4,2

Preceding crop during the wet season

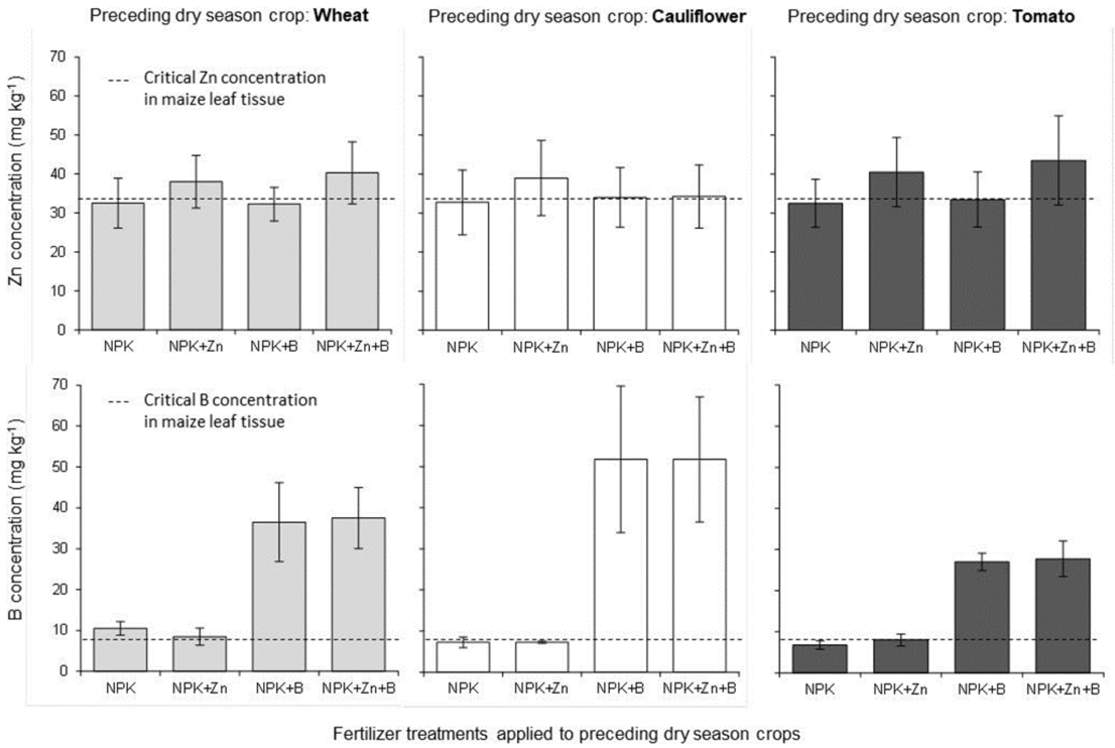


Figure 5.1: Residual effect of mineral fertilizer application to preceding crops on the leaf tissue concentration of Zn and B in non-amended maize (greenhouse experiment in Kathmandu, Nepal in 2018). No significant differences were observed between production systems and soil types. Thus, data are presented as means of two soil types (Acrisols vs. Fluvisols), two production systems (rice vs. maize as preceding wet season crops) and 4 replications (n=16). Dotted lines present the critical thresholds concentrations of Zn (<32 mg kg^{-1} *Rashid* and *Rafique*, 1989) and B (<9 mg kg^{-1} *Gupta*,2007) in maize leaf tissues.

5.3.3 Zinc and Boron uptake

Total uptake of residually available soil B and Zn was significantly affected by pre-crop fertilization, soil type, and production system, as well as interactions between all three factors (Tables 5.4,5.5). The uptake of soil Zn varied between 0.10 and 0.26 mg Zn pot^{-1} (untreated) and between 0.12 and 0.48 mg Zn pot^{-1} (pre-treated with Zn or Zn + B) (Table 5.4, Figure 5.2a). With previous Zn applications, the Zn uptake was always higher in the Acrisols (0.20-0.48 mg Zn pot^{-1}) than in the Fluvisols (0.12-0.24 mg Zn pot^{-1}), with a tendency for larger effects in the rice-based compared to the maize-based system (Table 5.4). Between vegetables, there was a slightly higher increase in Zn uptake after a tomato pre-crop, followed by wheat and cauliflower (Figure 5.2a).

B uptake by maize plants ranged from 0.02 to 0.08 mg B pot^{-1} (untreated) and from 0.05 to 0.48 mg B pot^{-1} (pre-treated with B or Zn + B) (Figure 5.2b and Table 5.5). In fact, all B fertilizers significantly increased the B uptake by maize, irrespective of the soil type, production system and type of pre-crop ($p<0.001$). Different from Zn, the largest residual effects were observed in the Fluvisols, compared to the Acrisols, for wheat and cauliflower pre-crops, with not much difference between rice-based and maize-based systems (Figure 5.2b). Only slight effects of soil type and systems were seen for tomato.

Overall, the magnitude of the residual effects of pre-applied Zn largely depended on the soil type and to some extent on the soil aeration status, while residual effects of B differed mainly as a function of the pre-crop and, to a lesser extent, of the soil type.

Table 5.4: Residual effect of Zn and B fertilizers applied to preceding dry season crops of wheat, cauliflower or tomato on the Zn uptake by non-amended maize, grown in potted soil of different origin (Fluvisols from a lowland and Acrisols from a mid-hill site) and from different production systems (rice vs. maize as preceding wet season crops) (greenhouse experiment in Kathmandu, Nepal in 2018).

Soil type	System#	Treatments	Zn uptake (g pot^{-1}) in greenhouse – Preceding dry season crops: Wheat			Cauliflower			Tomato		
Acrisols	Rice	NPK	0.2000	±0.0118	bc	0.2125	±0.0048	b	0.2600	±0.0211	c
		NPK+Zn	0.2400	±0.0082	b	0.3050	±0.0166	a	0.4800	±0.1025	a
		NPK+B	0.1575	±0.0126	d	0.2425	±0.0232	ab	0.3200	±0.0304	bc
		NPK+Zn+B	0.2850	±0.0182	a	0.2950	±0.0507	a	0.4300	±0.0383	ab
	Maize	NPK	0.2375	±0.0191	ns	0.2050	±0.0166	ns	0.1700	±0.0077	c
		NPK+Zn	0.3575	±0.0766		0.2525	±0.0259		0.2100	±0.0069	b
		NPK+B	0.2725	±0.0259		0.2225	±0.0266		0.1800	±0.0146	bc
		NPK+Zn+B	0.2900	±0.0257		0.2025	±0.0075		0.2500	±0.0159	a
Fluvisols	Rice	NPK	0.1600	±0.0014	b	0.1050	±0.0029	bc	0.1000	±0.0051	b
		NPK+Zn	0.2425	±0.0325	a	0.1475	±0.0063	a	0.1400	±0.0115	a
		NPK+B	0.1900	±0.0188	ab	0.1200	±0.0091	b	0.1200	±0.0039	a
		NPK+Zn+B	0.1525	±0.0164	b	0.1075	±0.0025	bc	0.1300	±0.0079	a
	Maize	NPK	0.1025	±0.0091	c	0.1075	±0.0075	b	0.1400	±0.0124	b
		NPK+Zn	0.1375	±0.0110	ab	0.1425	±0.0048	a	0.2400	±0.0322	a
		NPK+B	0.1525	±0.0047	a	0.1075	±0.0063	b	0.1600	±0.0183	b
		NPK+Zn+B	0.1500	±0.0092	a	0.1175	±0.0025	b	0.1700	±0.0079	b
Site/Soil			***			***			***		
System			ns			*			***		
Site x system			***			*			***		
Treatment			***			***			***		
Site x treatment			**			ns			**		
System x treatment			ns			ns			ns		
Site x system x treatment			ns			ns			ns		

Preceding crop during the wet season; x interaction between factors. Data values followed by the different letters within a column are significantly different by Least Significant Difference test (LSD) at p<0.05; ns = non-significant; * significant at p<0.05; ** significant at p<0.01 and *** significant at p<0.001. Zn concentration in prior crop: wheat: 32-40mg Zn kg^{-1}; cauliflower: 33-39 mg Zn kg^{-1}; tomato: 33-44 mg Zn kg^{-1}, ± standard error

Table 5.5: Residual effect of Zn and B fertilizers applied to preceding dry season crops of wheat, cauliflower or tomato on the B uptake by non-amended maize, grown in potted soil of different origin (Fluvisols from a lowland and Acrisols from a mid-hill site) and from different production systems (rice vs. maize as preceding wet season crops) (greenhouse experiment in Kathmandu, Nepal in 2018).

Soil type	System#	Treatments	B uptake in maize (mg pot^{-1}) – Preceding dry season crops: Wheat			Cauliflower			Tomato		
Acrisols	Rice	NPK	0.0475	±0.0029	c	0.0200	±0.0000	c	0.0399	±0.0032	b
		NPK+Zn	0.0325	±0.0011	cd	0.0375	±0.0025	c	0.0524	±0.0112	b
		NPK+B	0.0650	±0.0078	b	0.1675	±0.0170	b	0.2003	±0.0189	a
		NPK+Zn+B	0.1350	±0.0108	a	0.2725	±0.0484	a	0.1866	±0.0165	a
	Maize	NPK	0.0800	±0.0069	ns	0.0300	±0.0041	c	0.0290	±0.0013	c
		NPK+Zn	0.1100	±0.0224		0.0325	±0.0025	c	0.0378	±0.0013	c
		NPK+B	0.1300	±0.0126		0.0725	±0.0103	a	0.1457	±0.0117	a
		NPK+Zn+B	0.1100	±0.0096		0.0525	±0.0025	b	0.1162	±0.0074	b
Fluvisols	Rice	NPK	0.0600	±0.0005	c	0.0600	±0.0025	b	0.0400	±0.0021	d
		NPK+Zn	0.0800	±0.0101	c	0.0500	±0.0025	b	0.0700	±0.0055	c
		NPK+B	0.3600	±0.0363	a	0.3800	±0.0320	a	0.1400	±0.0043	b
		NPK+Zn+B	0.2500	±0.0267	b	0.3600	±0.0095	a	0.2200	±0.0130	a
	Maize	NPK	0.0425	±0.0036	b	0.0400	±0.0025	b	0.0300	±0.0022	c
		NPK+Zn	0.0225	±0.0018	b	0.0500	±0.0025	b	0.0400	±0.0056	c
		NPK+B	0.2900	±0.0090	a	0.4800	±0.0330	a	0.1500	±0.0169	a
		NPK+Zn+B	0.3000	±0.0181	a	0.4800	±0.0149	a	0.1100	±0.0053	b
Site/Soil			***			***			ns		
System			ns			ns			***		
Site/Soil x system			***			***			ns		
Treatment			***			***			***		
Site /Soi lx treatment			***			***			ns		
System x treatment			ns			ns			***		
Site /Soil x system x treatment			***			***			ns		

Preceding crop during the wet season; x interaction between factors. Data values followed by the different letters within a column are significantly different by Least Significant Difference test (LSD) at $p<0.05$; ns = non-significant; * significant at $p<0.05$; ** significant at $p<0.01$ and *** significant at $p<0.001$. B concentration in greenhouse: wheat: 10-38 mg B kg^{-1}, cauliflower: 7-52 B mg kg^{-1} and tomato: 7-28 mg B kg^{-1}, ± standard error.

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5.4 Discussion

The findings on key indicators such as biomass accumulation, plant uptake and tissue concentrations of Zn and B in non-fertilized maize underline the potential of using the residuals especially of B fertilizers applied to preceding crops for the follow-up crop maize. Even though Zn residual effects were less pronounced, they also consistently tended to increase – even though only slightly - biomass and Zn tissue concentrations of maize.

5.4.1 Residual zinc effects

Significant residual effects of previously applied Zn on maize dry matter were limited to those conditions, where Zn was applied to the pre-crop's wheat or tomato in the anaerobically managed (rice based) Fluvisols. At the same time, Zn uptake was higher in non-fertilized maize grown in soils with prior tomato and wheat cultivation, compared to cauliflower. These results are fully in line with the known high Zn demand of cauliflower (Singh et al., 2012), and the previously observed lower Zn uptake by wheat and tomato compared to cauliflower (Shrestha et al., 2020b).

In addition, a change from anaerobic conditions typical under wet season rice to aerobic conditions under the different dry season crops generally increased the Zn availability in the soil as well as the uptake by crops (Dai et al., 2016). Both effects combined may explain the overall somewhat larger residual effects of Zn application in the rice-based system. Several previous studies have shown similar residual effects of Zn applications to rice on succeeding crops such as wheat in Pakistan (Amanullah and Inamullah, 2016), Australia (Grewal and Graham, 1999), and India (Singh and Shivay, 2013; Kumar and Singh, 2018), maize-mungbean-rice rotations in Bangladesh (Hossain et al., 2008), or rice in Malaysia (Hafeez et al., 2013).

However, residual Zn effects were not sufficient to raise maize leaf Zn concentrations above the critical value of 32 mg Zn kg^{-1} (Rashid and Rafique, 1989) in the Fluvisols, irrespective of the pre-crop. This could be explained by the fact that the sandy Fluvisols of the lowland areas initially contained lower Zn contents (2.1 mg Zn kg^{-1}) compared to the clay-loam Acrisols of the mid-hill site (2.7 mg Zn kg^{-1}) (Shrestha et al., 2020a), which is in line with the fact that farmers in the mid-hills more regularly apply organic manures than farmers in the lowlands, resulting in higher soil nutrient contents (Tripathi and Jones, 2013). Thus, the sandy Fluvisols of the lowlands, which is also subjected to high rainfall during the monsoon season (Shrestha et al., 2020a) with associated nutrient leaching (Alloway, 2008), may require an addition of Zn at higher amounts than the currently recommended Zn fertilizer application rates to dry season crops, before significantly promoting growth and yield of the follow-up crops.

5.4.2 Residual boron effects

Even though the residual effects of B applications strongly increased B concentrations and B uptake of the follow up crop maize, increases in biomass were only minor. This is not surprising, since even without B applications, the B concentrations of the maize plants were only just below the critical B limit of 9 mg B kg^{-1} (Gupta, 2007). In the current treatments, where pre-crops were supplied with extra B, B concentrations in maize leaves reached higher, but not toxic levels. However, maize is a graminaceous species that is known for a relatively small B demand especially during vegetative growth (Lordkaew et al. 2001), and thus the relatively large excess B in the leaf tissue did not increase growth. In contrast, the B demand of maize increases during its development, and thus it could be expected that the residual effect of B treatments would become visible only at later growth stages, i.e. reflected in reduced final biomass or grain yield. It could also be assumed, that other follow-up crops with a higher B demand (e.g. dicot species) might benefit more from the residual B as actually observed in previous studies, where B applications to cabbage increased the B concentration and yield of both the cabbage (direct effect) as well as of the following bean crop (thus a residual effect - Gupta and Cutcliffe, 1984). Similar carry-over effects of pre-applied B have also been reported for wheat in Pakistan (Khan et al., 2006, 2011) and for oil-seed rape in rice-based rotations in China (Wang et al., 1997,1999), cauliflower in Bangladesh (Sarker et al., 2018), and rice in Malaysia (Saleem et al., 2011) as well as for corn in Iran (Aref, 2010).

Overall, different from Zn, the largest residual effects of B were observed after wheat and cauliflower in the Fluvisols, irrespective of the system (aerobic vs.

anaerobic) (Figure 5.2b). The latter observation is consistent with the chemistry of B, which does not change its availability depending on the redox status of the soil (Goldberg, 1997). Interestingly, residual fertilizer effects were much smaller with the pre-crop tomato. It should be kept in mind that cauliflower, which is known to have a rather high B requirement (Kotur, 1991), received here a total of 4.4 kg B ha^{-1} (corresponding to 157 mg B pot^{-1}) compared to 2.2 kg B ha^{-1} (or 79 mg B pot^{-1}) for tomato. The relatively low amounts of B applied to tomato were largely absorbed by the crop (Shrestha et al., 2020b), leaving little residual B left in the soil for inducing possible carry-over effects in maize. On the other hand, more B was left in the soil after wheat (with a very low B demand: Gupta, 2007; Wimmer et al., 2015) and cauliflower (with a larger supply), which could be taken up by the follow-up maize in the Fluvisols.

However, this residual B effect was not observed in the Acrisols. Currently, we can only speculate on the possible reasons for this interesting difference between both soils. Two options seem feasible: both soils used in this study reflect the typical low soil B availability that is commonly observed throughout Nepal (Shrestha et al., 2020a). Thus, the initial $CaCl_2$-extractable soil B of <0.3 mg B kg^{-1} (Shrestha et al, 2020a) was below the critical limit for growing cereals of 0.5 mg B kg^{-1} (Ahmed and Hossain, 1997; Gupta, 2007) in both soils. While the Fluvisols contained only 17% clay, this fraction amounted to 33% in the Acrisols (Shrestha et al. 2020a). It seems possible, that residual B not taken up by the pre-crops may have been adsorbed to some extent by the clay particles of the Acrisols and was thus less available for the succeeding maize. Alternatively, the fact that mid-hill soils (Acrisols) are conventionally more regularly supplied with organic manure might have resulted in a higher fraction of organic material in the Acrisols, which again could result in a higher adsorption of B and thus lower plant availability.

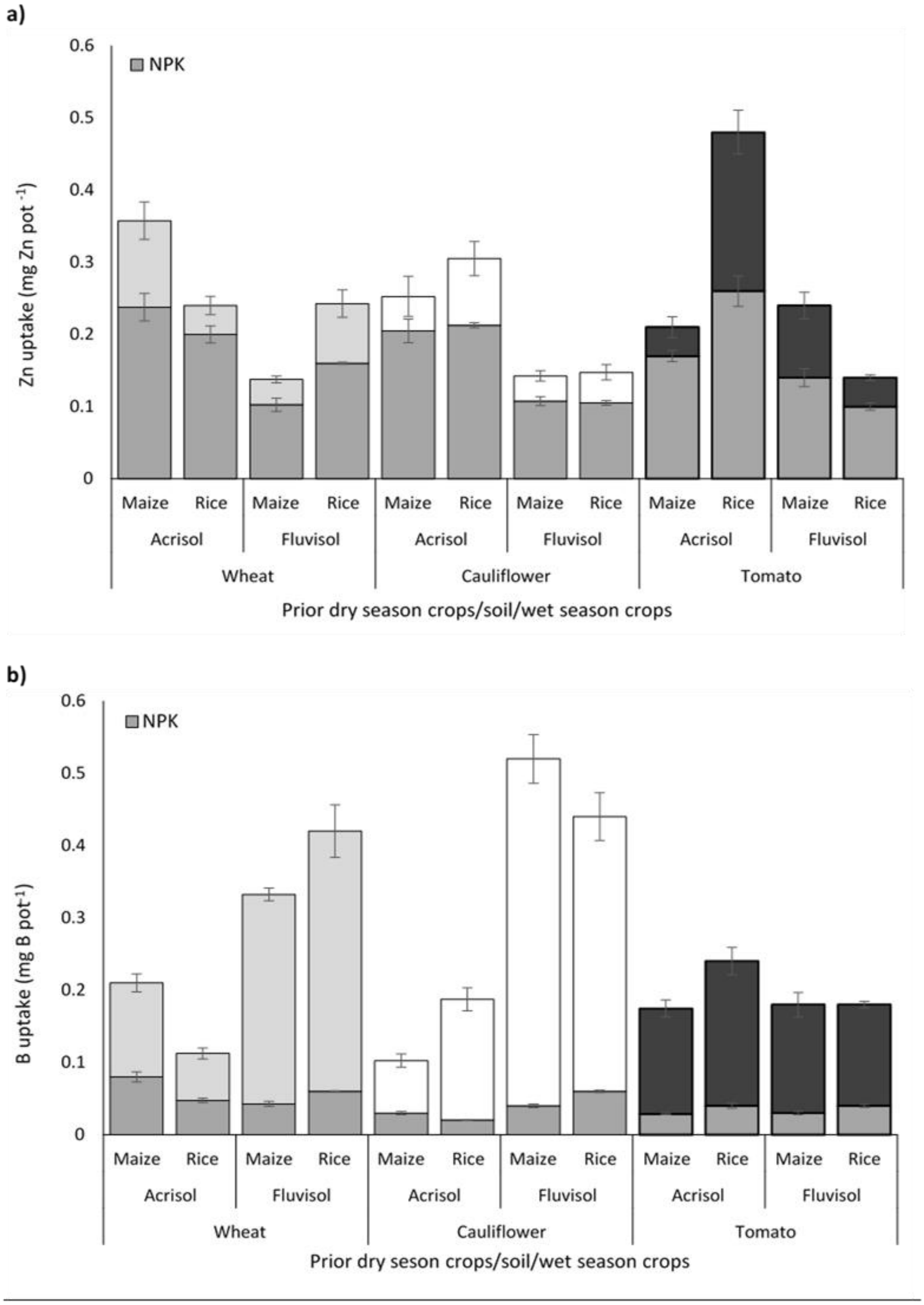

Figure 5.2: Residual effect of Zn and B fertilizers applied to preceding dry season crops on the Zn (a) and B (b) uptake by non-fertilized maize, grown in potted soil of different origin (Fluvisols from lowland and Acrisols from a mid-hills) and from different production systems (anaerobic soil in lowland rice or aerobic soil in maize during the wet season) a) represents means of NPK+Zn treatments, b) represents means of NPK+B treatments. Bars indicate standard errors of the mean (n=4).

5.5 Management implications and research needs

Collectively, our results indicate that residual effects of B and Zn fertilization of dry season crops benefitted subsequent wet season maize crop without additional micronutrient addition, at least at certain sites and after certain pre-crops. However, our results also clearly indicate that management recommendations have to be site specific, especially for pre-applied Zn, where the magnitude of the residual effects largely depended on the soil type and the soil aeration status. Soils inherently low in Zn, such as the Fluvisols of this study, which are also prone to significant leaching during the high rainfall monsoon season (Shrestha et al. 2020a), may consequently require higher amounts of Zn fertilization rates than currently recommended for the pre-crops, to maintain sufficient residual benefits for the subsequent crop. Farmers using the conventional rice-based system may benefit from higher residual Zn effects due to the repeated wetting and drying conditions, and thus higher Zn availability for the subsequent crop.

Overall, annual applications of expensive and often unavailable B and Zn fertilizers may not be required in the emerging vegetable-based cropping systems of Nepal. However, thus far we assessed only the residual effect for aerobically grown maize during vegetative development. Hence, long-term comparative assessments of residual effects in aerobic maize, flooded rice and vegetables under field conditions grown until maturity would improve our understanding of the dynamic and residual effects of these micronutrients in different cropping systems, and guide future site- and system-specific fertilizer recommendations.

Chapter 6

6 GENERAL DISCUSSION AND CONCLUSIONS

6.1 System shift and problem statement

The rice-wheat rotation is the most widespread cropping system in the Indo-Gangetic Plains (IGP) and the Himalayan mid-hills of South Asia, including Nepal. However, growing urbanization, combined with changing consumer preferences and recently increasing water shortages are driving the replacement of lowland rice with maize as well as of winter wheat by high-value vegetables such as cauliflower and tomato, especially in the vicinity of the urban centres. Cauliflower and tomato are the usually preferred vegetables among farmers and consumers. However, these crops are more nutrient-demanding than their predecessor crop wheat and hence the inclusion of these vegetables in current crop rotations will increase the demands of already deficient soil B and Zn. In addition, an aerobic cropping phase with maize cultivation, instead of lowland rice grown under anaerobic conditions, is likely to alter the availability of B and Zn in soils. Due to these recent changes, mentioned above farmers need to adapt their fertilizer application strategies to match changing soil supply and crop demands, bearing in mind that is should be technically feasible and financially viable. In anticipation of these critical change processes, this study addressed the site and system-specific B and Zn requirement and management both in the current rice-wheat and the emerging diversified vegetable-based rotations in Nepal.

Therefore, soil supply was assessed, as well as the direct crop response and the indirect residual effects on a subsequent non-fertilized crop at two sites in distinctly different agro-ecological zones with different soil types, and under both anaerobic (rice) and aerobic (maize) production systems. The response of traditional rice-wheat and the newly emerging vegetable-based systems to four fertilizer treatments (B, and Zn sole or in combinations) was assessed both under controlled conditions in potted soil in a sheltered greenhouse as well as in farmers' fields at the two contrasting production sites in 2017 and 2018. The findings of this research increase our understanding of the importance of the micronutrients B and Zn in deficient soils in Nepal, and lead to basis for providing recommendations of B and Zn fertilizer use in conventional and emerging cropping systems in Nepal and perhaps beyond such as entire IGP regions with similar agroecological conditions.

Besides the typical agronomic studies on biomass production, crop yield and nutrient uptake also potential economic effects were assessed, highlighting potential benefits of an adapted nutrient supply in emerging production systems both for income generation of smallholder farmers, and for improving regional food and nutritional security. The research findings have been outlined in three results Chapters, that were published/submitted in international peer-reviewed journals. The results are summarized in Table 6.1. They contribute to our understanding of B and Zn deficiency problems, of nutrient management requirements, of application effects on vegetables, and in regard to the way forward in terms of increasing food crop production in different growing environments of Nepal.

6.2 Soil B and Zn deficiencies are severe and increasing

Micronutrient deficiencies are associated recurrently with declining productivity of cropping systems throughout South Asia (Akhtar et al. 2013), more specifically, the wide-spread soil deficiencies of B and Zn in the IGP and Himalayan mid-hill regions are affecting the productivity of the conventional rice-wheat rotations in India (Nadeem and Farooq 2019) and the rice-wheat system (Karki et al. 2005; Andersen 2007) as well as the emerging vegetable-based systems in Nepal (Pokharel 2019). The reasons for B and Zn constituting a severe problem for regional crop production and associated food security are related on the one hand to the B and Zn deficient parent material of the agriculturally used soils, and on the other hand to socio-economic as well political specifics in the area.

Most soils in the region have derived from phyllite and schist resulting in the formation of often sandy soil with low to extremely low B and Zn contents (Hafeez 2013). The problem of severe soil B deficiency is even more wide-spread, concerning most of the production areas in the Himalayan mid-hills and a large share of adjoining lowland in Nepal’s IGP, making Nepal the world’s largest area of B deficient soils (Rerkasem and Jamjod 2004). The problem of low soil B and Zn contents concerns both the mid-hills and the lowland in Nepal, and both production environments and therefore were both addressed in this thesis research.

Table 6.1: The objectives, experiments, and associated major research results as well as a supporting visualisation of the findings.

Objectives	Experiments	Major results	Picture for visualization
Assessing the soil status of available B and Zn (**B and Zn diagnosis**).	• Pot experiment • Field validation experiment	• Observed clear visual symptoms of B and Zn deficiency in crops. • A decline in soil carbon and nitrogen under rice (anaerobic condition) to maize (aerobic condition) cropping system. • B and Zn deficient in soil. • Soil and plant Zn content was lower in the lowland compared to the mid-hills. • Crop yields of cauliflower and tomato was low.	  Cauliflower showed the B deficiency symptoms, pot experiment, Nepal, 2017
Determine the response of wheat and vegetables (cauliflower and tomato) to applied B and Zn (**B and Zn response**).	• Pot experiment • Field - validation experiment	• Zn applications increased wheat yields. • Applications of B (2.0-4.4 kg B ha^{-1}) alone or combined with Zn (3.3-4.4 kg Zn ha^{-1}) nearly doubled biomass accumulation, nutrient uptake and increased the economic yields (8 to >100%) of cauliflower and tomato. • Tomato and cauliflower growers would likely obtain benefits from Zn and B application whereas Zn would benefit wheat growers.	  Field experiment, lowland (left), +B treatment in cauliflower (right) Nepal, 2017
Analyze the possible carry-over effects of previously applied B and Zn on maize **(B and Zn residual effects)**	• Pot experiment	• Zn fertilization increased maize Zn concentration by 19-26% compared to the prior non-amendment pot. • B fertilization enhanced maize B concentration by 237-373% compared to non-fertilized maize. The largest B uptake of B residuals was observed in the Fluvisols, in lowland areas.	 Residual experiment, maize Nepal, 2017

The low concentrations of B and Zn at the study sites and the resulting critically low B (and Zn) concentrations in the biomass of wheat, but particularly of cauliflower and tomato were confirmed (see Chapter 3) in the diagnostic chapter of this thesis. Besides the critical concern of B and Zn deficiency, crop performance was affected by low and declining soil C and N contents also, which were further exacerbated by the introduction of an aerobic soil phase during the wet season, as a result of the current shifting towards water-saving of upland crop production systems (see Chapter 3).

The socio-economic and political factors exacerbating the B and Zn deficiency problem in Nepal relates in the first place to the fact that B and Zn fertilizer formulations are either not available on local rural markets or are not supplied but rarely available on time. On top of such distribution and availability challenges, most smallholders' farmers can presently not afford the relatively costly B and Zn fertilizers. The B and Zn deficiency lead to enhanced hunger by reducing crop yields and the Zn content of the edible plant parts -this lead to poor human health (Bhatt et al. 2020). Consequently, the combination of a severe and growing soil deficiency of B and Zn and of the non-availability and non-affordability of B and Zn fertilizers make Nepal a suitable study case for B and Zn fertilizer response studies and experiments that may influence both the public and private sector investors to address this key issue in the near future.

6.3 Application of B and Zn is essential for sustainable crop production in Nepal

As the B and Zn demand is currently high and increasing with progressing system shifts, the effects of externally-applied B and Zn on crop performance attributes were assessed (see Chapter 4). Even though only small quantities of B and Zn are required, these two micronutrients are nevertheless essential for crop growth and production (Gupta 2007; Broadley et al. 2012), whilst furthermore the demand for these micro nutrients increases with the inclusion of horticultural crops such as cauliflower and tomato in the crop rotations (Wimmer et al. 2015). The crop responsiveness to B application, either alone or combined with Zn fertilizers, regarding biomass production, B uptake and economic yield was more pronounced in cauliflower and tomato than for wheat in both pot and field conditions, irrespective of the soil or production system (see Chapter 4). Previous studies have also confirmed B fertilizer increased the production of cauliflower (Ahmad et al. 2012; Ribeiro et al. 2017) and tomato (Davis et al. 2003) in North Carolina United States.

An application of Zn fertilizers increased the wheat yield. Furthermore, the beneficial effect of Zn supply alone on vegetable crops was not as pronounced as the effects of additional B supply (see Chapter 4). It has been repeatedly underlined that soil B (Bell and Dell 2008; Goldbach 2020) and Zn are essential minerals for crop production (Cakmak et al. 1996; Dobermann and Fairhurst 2000). Hence when considering the expected population growth and ongoing developing urbanization in Nepal adding the micronutrients B and Zn are obligatory in the newly diversified cropping system in the entire IGP region (Srivastava et al. 2000; Rerkasem 2002; Nadeem and Farooq 2019). The findings showed that overall, economic yield of cauliflower and tomato grown in the mid-hills was higher than at the lowland sites, however, the relative yield increases to applied B and Zn fertilizers were more pronounced in sandy soils dominating in the lowlands (Chapter 4). Despite the known perils of using pot experiments as the basis for extrapolation to field and farmer conditions, the current findings (see Chapters 3 and 4) underline the importance of applying an adequate dose of B and Zn amendments to the emerging vegetable-based cropping system in Nepal to ensure increased and sustainable crop production and has the potential of consequently improve farmers income (see Chapter 4) as well as improved food security.

6.4 Applications of B and Zn fertilizer are potentially financially viable

Vegetable farming is increasingly gaining importance and rapidly replacing the traditional winter wheat crop near urban centres and in areas with good market access-it is potential becoming the major source of income for peri-urban farmers in Nepal. Plants require small fractions of the micronutrients B and Zn only, but a deficiency of these elements usually stunts plant growth and reduces yields, particularly in vegetable crops. Hence Zn fertilization at the rate of 3.3 Zn kg ha^{-1}, improved the yield of wheat (see Chapter 4).To this end, low to moderate applications to cauliflower and tomato of between 2.0 and 4.4 kg B ha^{-1} and 3.3-4.4 kg Zn ha^{-1} alone or combined nearly doubled biomass accumulation, nutrient uptake and increased economic yields up to >100% (Chapter 4) in both pot and field validation experiments. Soil B amendments not only increased the crop yield but also improved the quality of cauliflower (Solanki et al. 2018) and tomato (Davis et al. 2003; Huang and Snapp 2004, 2009) as a result, financial benefits to the farmers was increased (Wani et al. 2016; Chander et al. 2019). At current price levels of B and Zn fertilizers, and the market price of the harvest products in Nepal would be a significant benefit when adopting these technologies and innovations (see Chapter 4) in new cropping system; a farmer would not lose her/his investments in Zn and B fertilization. The result revealed that the monetary gain of investing in Zn fertilizer was profitable in wheat, but only marginal in the vegetables such as cauliflower and tomato while additional investments in B fertilizers was highly profitable, irrespective of the soil type or cropping system (Chapter 4, section 4.3.3). In addition, the application of B and Zn fertilizers in cauliflower and tomato showed substantial positive carry-over effects (i.e. biomass and nutrient concentration) on the follow-up crop maize.

6.5 Application of B and Zn cause beneficial residual effects on subsequent crops

Soils in Nepal have predominantly low to medium soil fertility (Bajracharya and Sherchan 2009). Subsequently applications of B and Zn are required to promote sustainable plant growth and development. However, currently, B and Zn fertilizers are not easily accessible to, and affordable by rural farmers in Nepal, but the findings of this research provide strong evidence to support promoting B and Zn additions and encouraging the private or public sectors to increase their efforts in making such fertilizers available and accessible to the farmers - the combined, direct effects on the dry season crops and the indirect effects on the follow-up wet season crop maize are clear (Chapter 4 and 5).The levels of residues that remain after applying B and Zn to previous crops were sufficient to balance out the existing deficiencies in subsequent wet season maize (Chapter 5).The previous findings also confirmed the beneficial residual effects of B (Wang et al. 1997; Rashid et al. 2007) and Zn additions (Grewal and Graham 1999; Gupta and Kalra 2006) on succeeding crops.

The magnitude of the residual effects of pre-applied Zn depended largely on the soil type and soil aeration status which was highly significant on Acrisols (mid-hills), but of much lower in Fluvisols (lowland). The sandy Fluvisols of the lowland areas initially record lower Zn contents compared to the clay-loam Acrisols of the mid-hills; the latter, however are also prone to nutrient leaching, during the high rainfall in monsoon season, and may consequently require higher amounts of Zn fertilization rates than the currently recommended doses for the pre crops, in order to maintain sufficient residual benefits for the subsequent crop. While residual effects of B differed mainly according to the previously cultivated crop type but was high in cauliflower in the Fluvisols in the lowland. The regular application of B-fertilizers can induce a toxic effect in soils (Gupta et al., 1985), although this is dependent on the crop type, and the location (Rashid and Ryan 2004); it is also dependent on the growing season- B transport to the leaves is higher in the humid hot season but lower in the cool-season (Goldbach 2020). It is also known that wheat demonstrates low B uptake (Reid 2013) while tomato and cauliflower are expected to be more tolerant of B toxicity (Goldbach 2020). Annual applications of costly and, in Nepal, often unavailable B and Zn fertilizers to all crops in the rotation may not be required. Yet, given the narrow range between B deficiency and toxicity, the speed of translocation of B has a profound effect on the B deficiency and toxicity in crops (Brown and Shelp 1997). The findings of this research support reducing the potential of toxic effects of adding micronutrients in the cropping systems and enhancing environmentally friendly and cost-effective production.

6.6 Conclusions and outlook

Soils deficient in B and Zn are extensive in Nepal, and the ongoing system shift in cropping has altered the availability of soil nutrients which is evidenced in the substantial increase in the demand for B and Zn in the new cropping system. An application of Zn fertilizers increased wheat production and tackling the B and Zn deficiencies has become essential and is highly profitable in cauliflower and tomato production. Furthermore, the residual effects of application of B and lesser extent Zn to preceding crops were nevertheless still adequate to overcome the current deficiencies in subsequent wet season maize. The overall findings increased the understanding of B and Zn requirements for dry season crops such as wheat, cauliflower, and tomato. The site and system-specific nutrients management basis for the study was appropriate and can ultimately contribute to improved soil fertility management for sustainable agriculture production in Nepal and perhaps even beyond such as in IPG regions.

A major task for Nepalese public sector supporting the agricultural sector is to formulate recommendation for appropriate site-specific doses of B and Zn fertilizers; these recommendations, in the longer term, must be based on more intensive and long term field studies to obtain a better understanding of the dynamics of these micronutrients in order to formulate appropriate doses for different soil types and cropping systems.

In the current study, analyzed the above-ground dry biomass plant sample in the early reproductive stage of the crop; this could be considerably enhanced, however, by the analysis of plant edible parts (e.g. grains, fruits and curds) to improve the current understanding of nutrient content, particularly Zn, in harvested and consumed products. This could be crucial for human, especially children and women health and improving the food and nutrition security of Nepal.

The demand for B and Zn fertilizers is likely to dramatically increase over the medium term with the further expansion of urban areas and the associated shifts in production systems in the country. The great majority of farmers are unaware of the wide-spread visual symptoms of B and Zn deficiencies. Without the identification and adoption of proper techniques to address the B and Zn deficiency problems, the ongoing shift in production systems will not be sustainable. In addition, the farmers will fail to meet the increasing and more discerning demands of urban consumers, and their own financial expectations. There is thus an urgent need for both public and private sectors to increase farmers' awareness of soil B and Zn management for increasing production in a sustainable way. The private sector, national and international fertilizer traders and agencies, are required to take the on-going system shifts as an opportunity to produce suitable and affordable B and Zn fertilizers perhaps even with the site- and crop-specific packaging, for supply these within local and regional markets. Furthermore, these findings are expected to be beneficial not only for farmers but also for researcher, extension workers and policymakers alike, and if the conclusion are properly recognized by the stakeholders, will increase the crop production, decrease food imports to Nepal and curb the growing food insecurity in the IGP regions. These research findings will also contribute to the knowledge of these crop nutrient deficiencies in the region; the soils of south and east of China, as well as India and Bangladesh are commonly B deficient (as are the soils in Brazil and Japan). Soils deficient in Zn also occur in India, Pakistan, Bangladesh, and Sri Lanka. The findings of this research could therefore have a significant and beneficial impact on both productivity and human health in the region and beyond.

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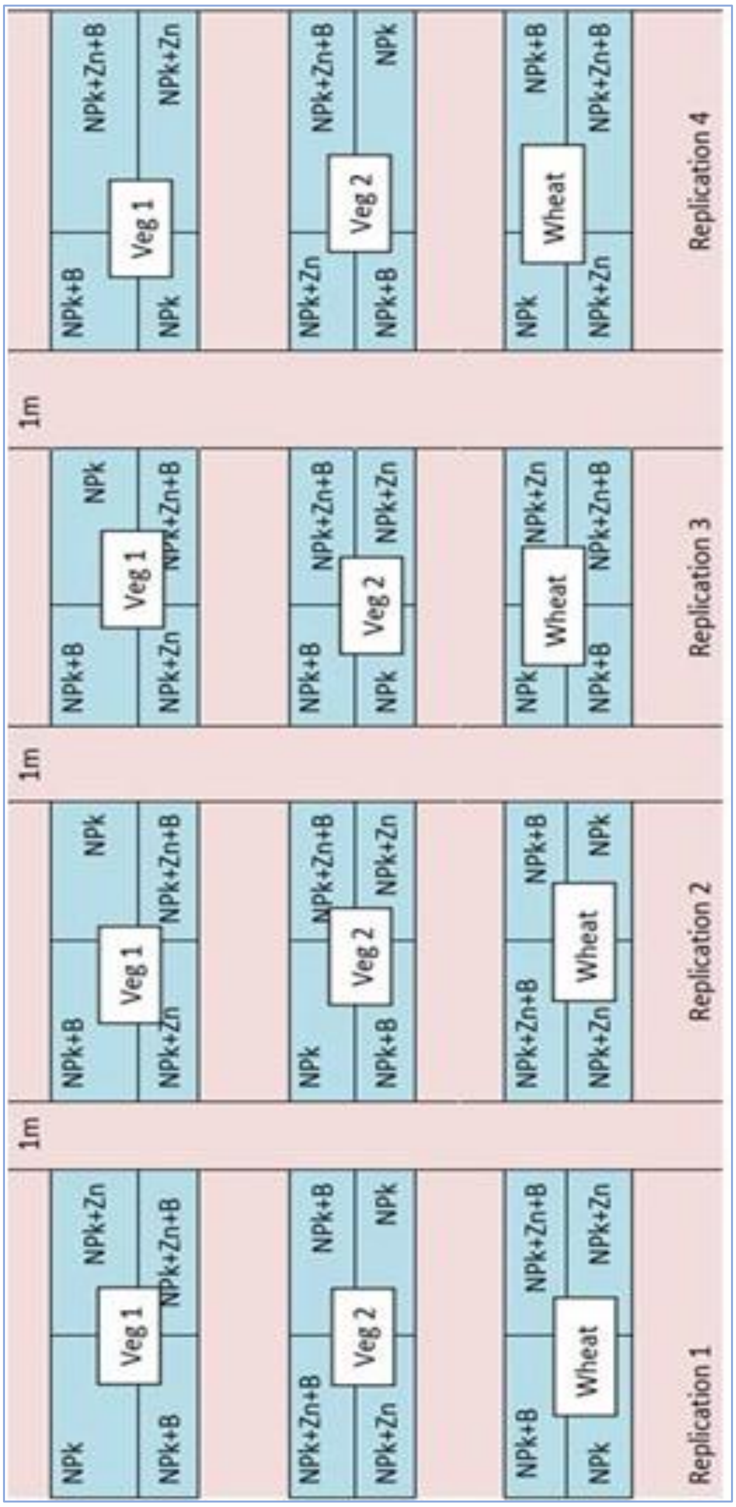
8 APPENDICES

Appendix A1

Field experimental layout



Appendix A2

Views from the field experimental sites

Photo 1: Maize field, mid-hills (Kavre), Nepal 2017

Photo 2: Rice field, mid-hills (Kavre), Nepal 2017

Appendix A3

Views from the greenhouse

Photo 3: Wheat growing in pots, greenhouse experiment, Nepal 2017

Photo 4: Cauliflower growing in pots, greenhouse experiment, Nepal 2017

Photo 5: Tomato growing in pots, greenhouse experiment, Nepal 2017

Photo 6: B deficiency symptoms showed in cauliflower, greenhouse experiment, Nepal 2017

Appendix A4

Views from the field experiment

(a) **(b)** **(c)**

Photo 7: B deficiency symptoms (hollow stem with brownish necrosis and brownish curds) showed in cauliflower (a) and (b), and B sufficient (c) field experiment, mid-hills (Kavre), Nepal 2018

Photo 8: Field-grown severely B-deficient tomato fruits with deformation and corky, broken, and necrotic epidermal tissues (left) And B-sufficient tomato (right) at the lowland site in Rampur, Nepal 2018

Photo 9: Cauliflower, field experiment mid-hills (Kavre), Nepal 2017

Photo 10: Experiment plot, lowland (Rampur), Nepal 2018

Photo 11: Field-grown B-deficient cauliflower; maize field, mid-hills (Kavre), Nepal 2018

Photo 12: +B treatment cauliflower, maize field, mid-hills (Kavre), Nepal 2018

Appendix A5

Photo 13: Maize field at the mid-hills in Kavre (upper), rice field in Kavre (middle)and maize field at the lowland site in Rampur, Nepal 2018

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