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Opportunities and challenges of managing the rhizosphere's biota – for food intensification, through controlled application of fertilisers with commercial Arbuscular Mycorrhizal fungi

Transdisciplinary perspective

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Abstract

Protecting the environment and halting the depletion of natural resources - while intensifying food yield to feed a growing global population, require alternatives to excessive application of synthetic fertilisers and pesticides. Interdisciplinary holistic research, complementary to discovery science, is needed to explore linkages between relevant, but different, knowledge domains and constituencies of crop production. This study explores the opportunities and challenges of managing the rhizosphere's biota through controlled application of fertilisers and mycorrhizal fungi, from a technical point of view linked to several environmental and socio-economic factors governing the agricultural system. It highlights the need for dynamic soil modelling and research, systematic food-wastage management and integrated farming approaches as potential strategies for sustainable intensification of food production. The role of earthworms as soil 'engineers', in relation to mycorrhizal fungi and the C-N equilibrium is also highlighted. A laboratory experiment was conducted over six weeks to examine plant growth and P availability in the rhizosphere, in the presence of mycorrhizal fungi and 2% worm cast. The treatment which received the combination of both organic fertilisers and conventional NPK application resulted in more than 137% fresh-weight enhancement, or 91% dry biomass enhancement in compared to the control. There was also a significant enhancement in P availability in the rhizosphere in the treatment with the P-efficiency capsule, specially designed for this experiment. Roots stained with Trypan Blue were examined microscopically to observe arbuscular mycorrhizal colonisation, and colonisation was found in all treatments, in more or less visible abundance. Data on social aspects of food production and soil nutrients were gathered from peer-reviewed literature, relevant popular Google searches, reliable media sources, popular YouTube educational videos, social media, and surveying 20 allotment-holders in the Edinburgh area. Data indicated an appreciation of legumes as a basic crop by 100% of allotment-holders surveyed, but less awareness of the value of biological inoculants such as

mycorrhizal fungi, in efficient soil management. Overall, the dissertation stresses the importance of completing the food production loop production through managing nutrient flow in the rhizosphere component of soil ecosystems.

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(By first author - August, 2016)

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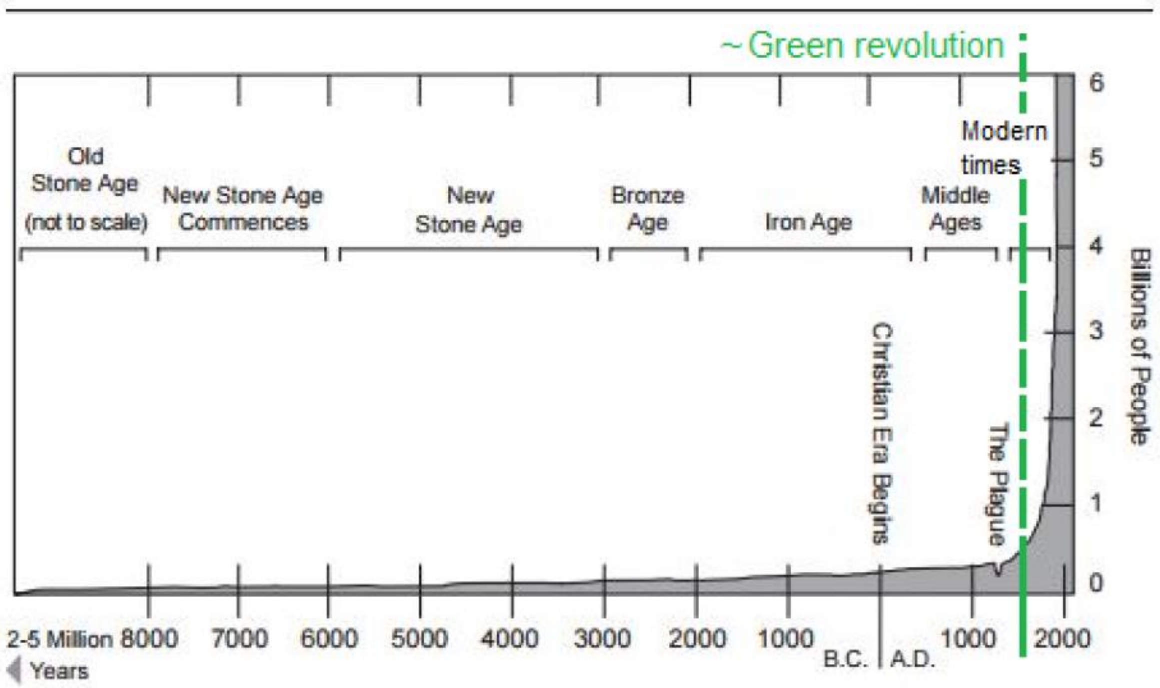
CHAPTER 1

Food intensification, past and future

The green revolution, which occurred between the 1930s and late 1960s, brought changes in agronomy, cereal varieties, inorganic fertilisers, and irrigation, and saw the introduction of new pesticides. It resulted in huge changes, mostly in developed countries (Hazell, 2009), and has been a major contributor to global growth in the human population, currently 7 billion (The Royal Society, 2009). Although growth has slowed since 1960, it is still projected to hit 9.1 billion by 2050. If current agricultural and consumption trends continue, by 2050 the world will need to increase food production by 50% to accommodate for global needs for calories and nutrients (FAO, 2011).

Global calls for food intensification are based on current and projected population growth, current consumption rates and hunger rates (Bommarco, 2013; Garnett and Godfray, 2012; Scharntke et al., 2012; Tilman, 2011). Less stressed, however, is the fact that current population growth is, itself, the outcome of successful but probably unsustainable food intensification (The Royal Society, 2009). The invention of agriculture some 10,000 years ago, enabled people to co-create food rather than forage, thereby allowing human communities to flourish. Now, in the wake of the industrial revolution, fuelled by cheap fossil fuel (Pfeiffer, 2006 cited in Cordell, 2010), agronomy, science and technology have triggered unprecedented population growth (Figures 1.1, 1.2) with much inequality between industrial and developing countries, and more need of mass food-production. In the 19th century, the yield of English wheat increased from 0.5 to 2 t/ha, but then reached 7 t/ha by the end of the 20th century (Hazell, 2009). Similarly, gross world food production grew from 1.84 billion tonnes since 1961 to 4.38 billion tonnes in 2007, an increase of 138% (The Royal Society, 2009), and that increase was achieved mainly by improved agronomy and the development of inorganic fertilisers (Hazell, 2009).

In 1961 the FAO estimated average global calorie availability per capita to be 2,193 kcal/day. By 2011, it had reached 2,868 kcal/day (FAO, 2015) with an alternative estimate for 2003-2005 of 3050 kcal/day (FAO expert paper). The global average daily calorie availability should, according to the FAO, reach 3,050 kcal per capita – comprising a 40% (about 900^6 tons) increase in cereals production alone. At the time of writing, however, there are about 700 million people, mostly in developing countries, whose calorie intake is insufficient to meet their daily physiological needs (WFP). Although the hunger described by thoses statistics is concentrated in developing countries, it is not a localized issue. During the High Level Political Forum for Sustainable Development (HLPF, 2015), developed countries agreed to dedicate 0.7 of their annual GDP as aid to overcome hunger and poverty in developing countries by 2020 (UN, 2015).



Slightly modified from

Salk, P. (1992) An Evolutionary Approach to World Peace.

Figure 1.2. Population growth in relation to agricultural development.

Whether this level of yield increase, to keep pace with current and (growing) future demand, will be attainable, is debatable. There are political, physical, economic, health and environmental challenges to such intensification: eradicating hunger and extreme poverty, forexample, is expected to require $276 billion annually (FAO, 2014). The current projection - requiring 40-50% increase in food production by 2050, does not take into account losses in yield and available lands through wastage, inefficient resource allocation and environmental degradation (UNEP, 2009). Agriculture is much more than just crop cultivation. Rather, it is a complex system comprising social, economic, environmental and political components (Figure 1.3), and must be addressed more holistically (Savory, 1991; Royal Society, 2009).

There are, however, difficulties inherent in the holistic approach. It does not provide straightforward answers to sustainability challenges and requires complex modelling rather than one-dimentional experimenting. Additionally, it requires acceptance of some uncertainty arising from the complexity (Savory, 1991). The approach, as first proposed by Savory (1991), focuses on the economic and environmental aspect of sustainable land management, but does not adequately highlight the political and social hurdles to such management. In other words, it refers to humankind by the pronoun ‘we’, as if today's combined human population were capable of making a unified synergetic and binding political decision.

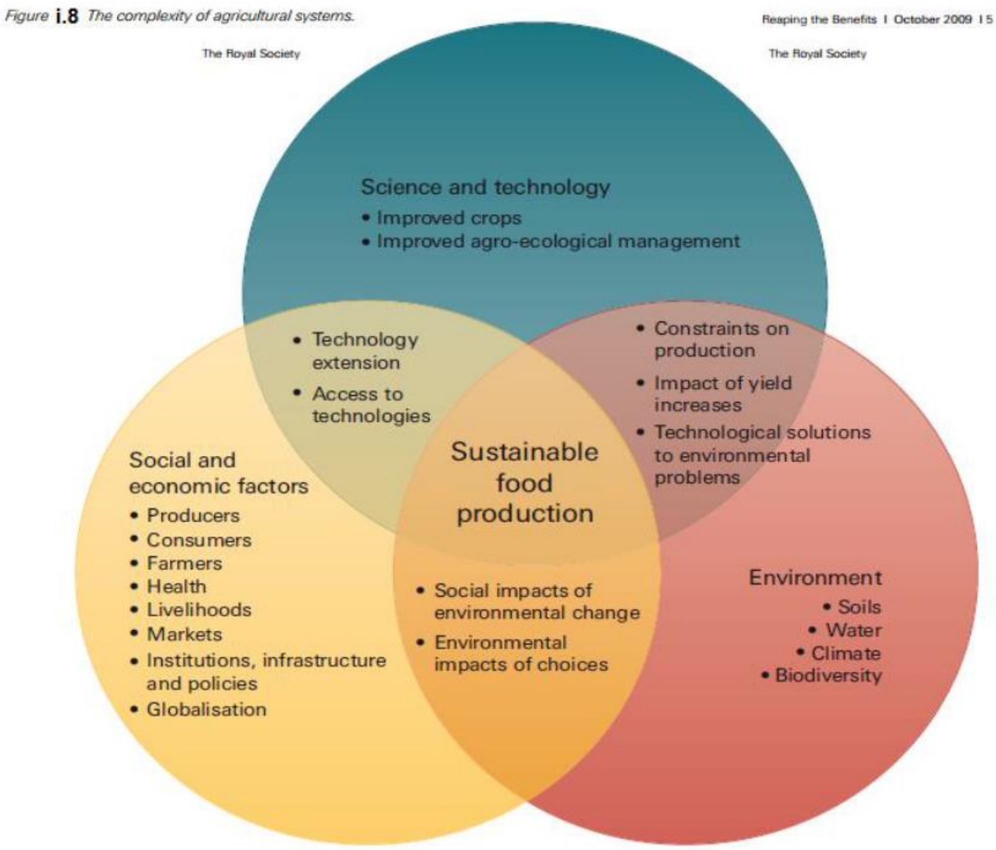


Figure 1.3. The complexity of the agricultural system (Royal Society, 2009).

Agriculture imposes enormous pressures on ecosystems. Many natural resources worldwide have already displayed heightened signs of degradation. The Millennium Ecosystem Assessment reported that 15 out of 24 of the ecosystem services it recognizes have been exploited un-sustainably. Intensification of food production (high-input-high output) has caused loss of soil minerals, biodiversity and aggregation, and has resulted in desertification, deforestation, pollution of water, diminution of many genetic resources, and an overall deterioration of ecosystems (FAO Expert Papers). Roughly one third (35% in weight, 24% in calories) of all food produced for human consumption each year (using, incidentally, 24% of all freshwater) is either lost or wasted (FAO, 2011, Table 1.1), and wastage in developing countries has a different profile from that in developed countries (Figure 1.4). Inefficient use of fertilisers is another form of nutrient loss not well acounted for.

Table 1.1. Average global food losses and waste according to FAO, 2011

30%	20%	35%	45%	20%	20%	45%
Cereals	Dairy	Fish and seafood	Fruits and Vegetables	Meat	Oil seeds and pulses	Roots and tubers

The biggest challenge today, therefore, is not how to increase humanity's food production (as is sometimes claimed), but to reduce food wastage and decrease the food production footprint. That challenge can be met. Setting targets to reduce food waste, developing protocols to regulate management of food waste/loss, encouraging waste-management innovation (UNEP, 2009) and, above all, enhancing agronomical precision can draw food accessibility towards the 2050 target without significantly changing input.

Efficient management of soil biota through organic practices (principally recycling waste food waste which is no longer consumable), reduces the need for inorganic fertilisers, pesticides and fungicides. In addition to enhancing soil aggregates and overall soil structure, it is also potentially capable of cutting down GHG emissions and decreasing pollution of water resources

(Abbott, 1991; Arancon et al., 2006; Blum, 2005; Giri & Mukerji, 2004; Sullia, 1990; Tarafdar & Praveen-Kumar, 1996).

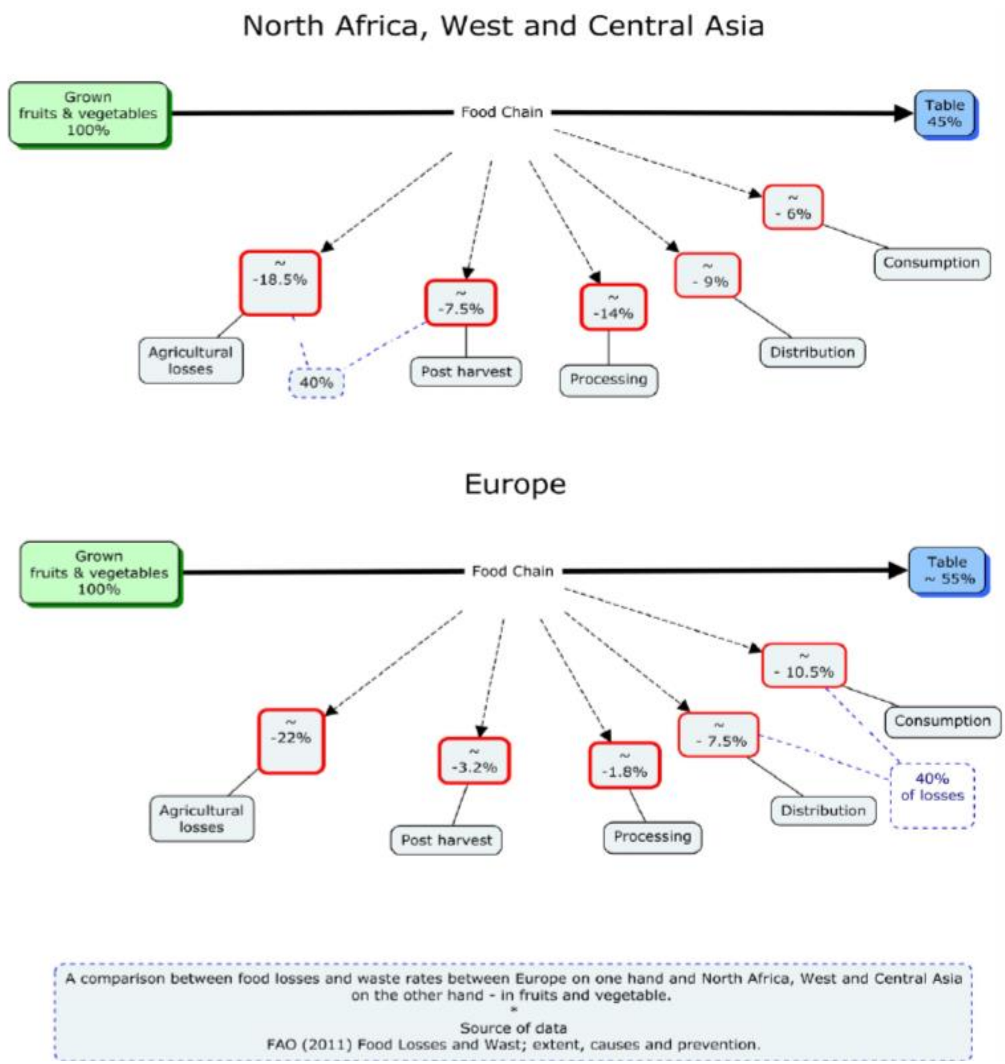


Figure 1.4. Comparison of food wastage profiles in developing and developed regions.

Crop production techniques which avoid the use of soil, vertical agriculture, urban agriculture, genome editing, and precision applications may all enhancing the efficiency of crop production (Business Insider, 2004), but food waste/loss management remains the missing link in the food system. Adding that missing link would complete the food system's cycle, turning the linear model of a food chain into a circular regenerative viable production model.

This does, however, requires the holistic (Ringler, 2016) precision (Nolan et al., 2009) approach to food production in its relation to energy, governance and innovation (FAO, 2014; Stockholm Environment Institute, 2011; The Royal Society, 2009). To feed the world, rather than a 50% increase in food production, what is most needed is systematic retrieval of nutrients lost through unavoidable food wastage, using good soil management practices, composting, vermicomposting, integrated farming approaches and balanced governance.

CHAPTER 2

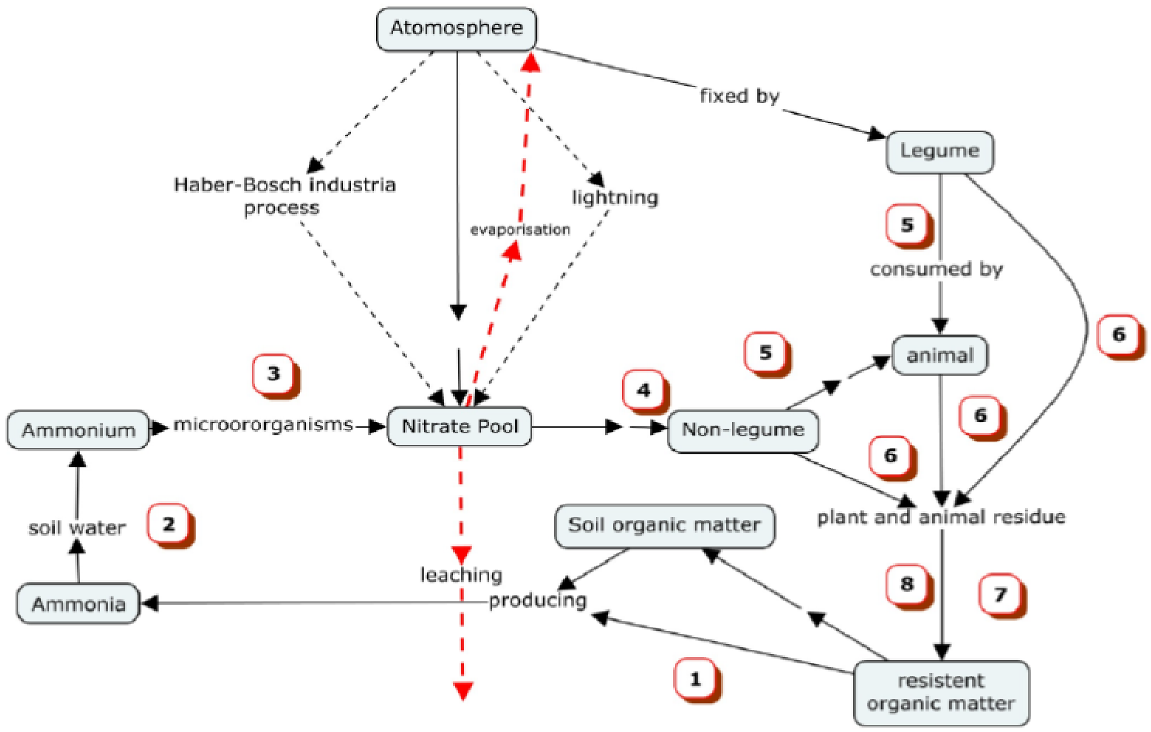
Soil fertility between organic and inorganic amendment

2.1. Synthetic fertilisers: a historic outline focusing on N and P

Earliest forms of agriculture, some 10,000 years ago, relied entirely on the soil's spontaneous bounty. About 5000 years later, the practice of adding organic fertlisers began. That enriched the soil, compensating for plant uptake and nutrient removal. The ancient Egyptians, Romans, and early Germans were all recorded to have applied animal manure and wood ash to agricultural soil. In the 1800s, huge deposits of guano were discovered on islands off the coast of South America. Guano is the solidified dung of seabirds, accumulated over thousands of years on their island colonies. It typically contains 8-16 % N, 8-12 % equivalent phosphoric acid, and 2-3 % equivalent potash (Paul et al., 2012). Use of guano became widespread for a while, not only as fertiliser, but also as a source of nitrates for production of explosives, but supplies were rapidly exhausted (Scherer, 2000).

2.1.1. Nitrogen

The largest single component of the earth's atmosphere (approximately 78%) is nitrogen (N). N is assimilated naturally into the earth's soil and water by a process called fixation. Fixation may be abiotic, for example through the electrical discharge of lightning, or biotic, through the activity of various living organisms, most commonly through microbial symbionts of legumes, the most important being members of *Rhizobium* and related bacterial genera which stimulate root nodulation of legumes (Kinkema et al., 2006). There are also some less common actinorhizal symbionts such as members of the bacterial genus *Frankia* (Pawlowski, 2009) and associations with other non-leguminous plants such as, for example, alders (Kennedy and Tchan, 1992). Further biotic nitrogen fixation occurs through cyanobacteria (Latysheva et al., 2012) working on decomposed crop residues and animal manures (Figure 2.1).



Source:
Zhang, H. and Raun, B. (2000;2006) Soil fertility Handbook.
Published by Department of Plant and Soil Sciences.
Oklahoma Agricultural Experiment Station. Oklahoma Cooperative Extension Service.
Division of Agricultural Sciences and Natural Resources. Oklahoma State University. Figure 2.4, pp. 16.
Available at [http://soiltesting.okstate.edu/extension-fact-sheets/SFHB2006C.pdf] accessed on 13/07/2016.

Figure 2.1. Soil nitrogen cycle; organic and inorganic.

The nitrogen added to the soil naturally has been estimated at 3 to 5 kg/hectare/yr. This constitutes about half of the amount required for agriculture to meet current global needs. The other half comes from the (r)evolutionary process of synthesising ammonium from atmospheric N using a technique invented in the early 1900s by Haber and Bosh (Smil, 1999; Zhang and Raun, 2000). Very little of that N actually reaches the human end-user in the form of food: only 14% of N applied to agricultural land as a fertliser (Galloway and Cowling, 2003) and only 28% of N used for animal production (Figure2.2).

A comprehensive literature review showed that N (as NH_3, together with sulphur dioxide (SO_2) are major acidifying pollutants which affect soil, ground and surface waters and biological diversity (Seppälä et al., 2006; Tuomistoa et al., 2012): in Europe, for example, agriculture, specifically livestock production, accounts for about 80% of all NH_3 emissions (EMEP, 2008 cited in Tuomistoa et al., 2012).

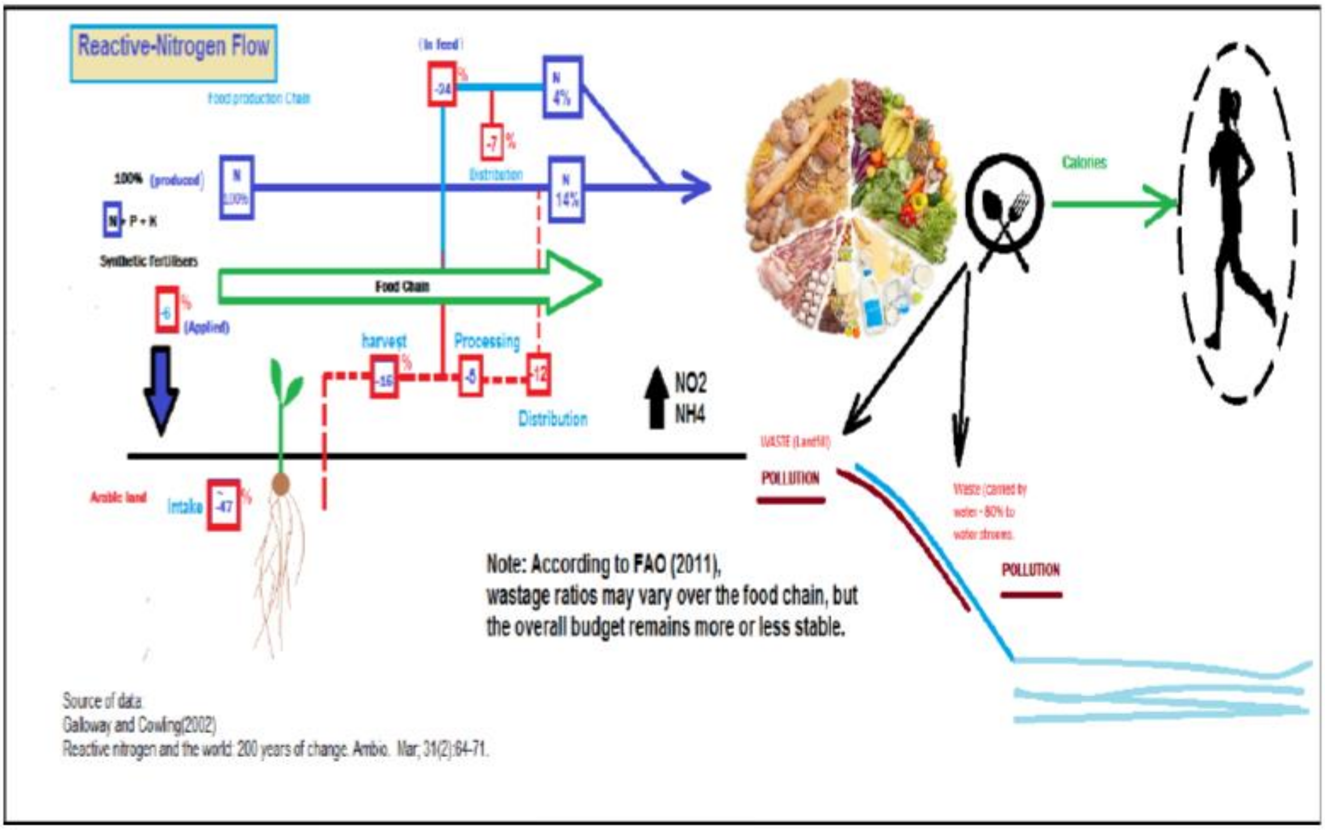


Figure 2.2. Nitrogen fertiliser in the food chain (illustration created for this study).

2.1.2. Phosphorus

> "We may be able to substitute nuclear power for coal power, and plastics for wood... but for phosphorus there is neither substitute nor replacement." Isaac Asimov, "Life's Bottleneck", Fact and Fancy.

With the rapid exhaustion of guano as a source (Wolfe, 2001), phosphate fertlisers currently come mostly from mining and treating phosphate-bearing rocks with sulphur-based acids

(Kauwenbergh, 2010). Production from natural organic sources has become of a minor commercial significance, because of the high cost per unit and the scarcity of supply.

Although phosphorus is a major limiting nutrient for plant growth, it is the least readily available in soil for plant uptake. P soil tests don't measure P abundance in agronomical soil, but provide an index of exchangeable-P availability. This is because P is highly reactive, adsorbing readily to other compounds present in the soil; such as calcium (Ca), aluminium (Al) and Iron (Fe), and forming a sort of P soil stock as it becomes temporarily unavailable for plant uptake. The strength of P compound bonds (i.e. their stability) are highly dependent on soil pH level (Zhang and Raun, 2000). In a neutral to high pH, calcium phosphates form, while in low pH (acid soils), Fe and Al phosphates are produced (Figure 2.3).

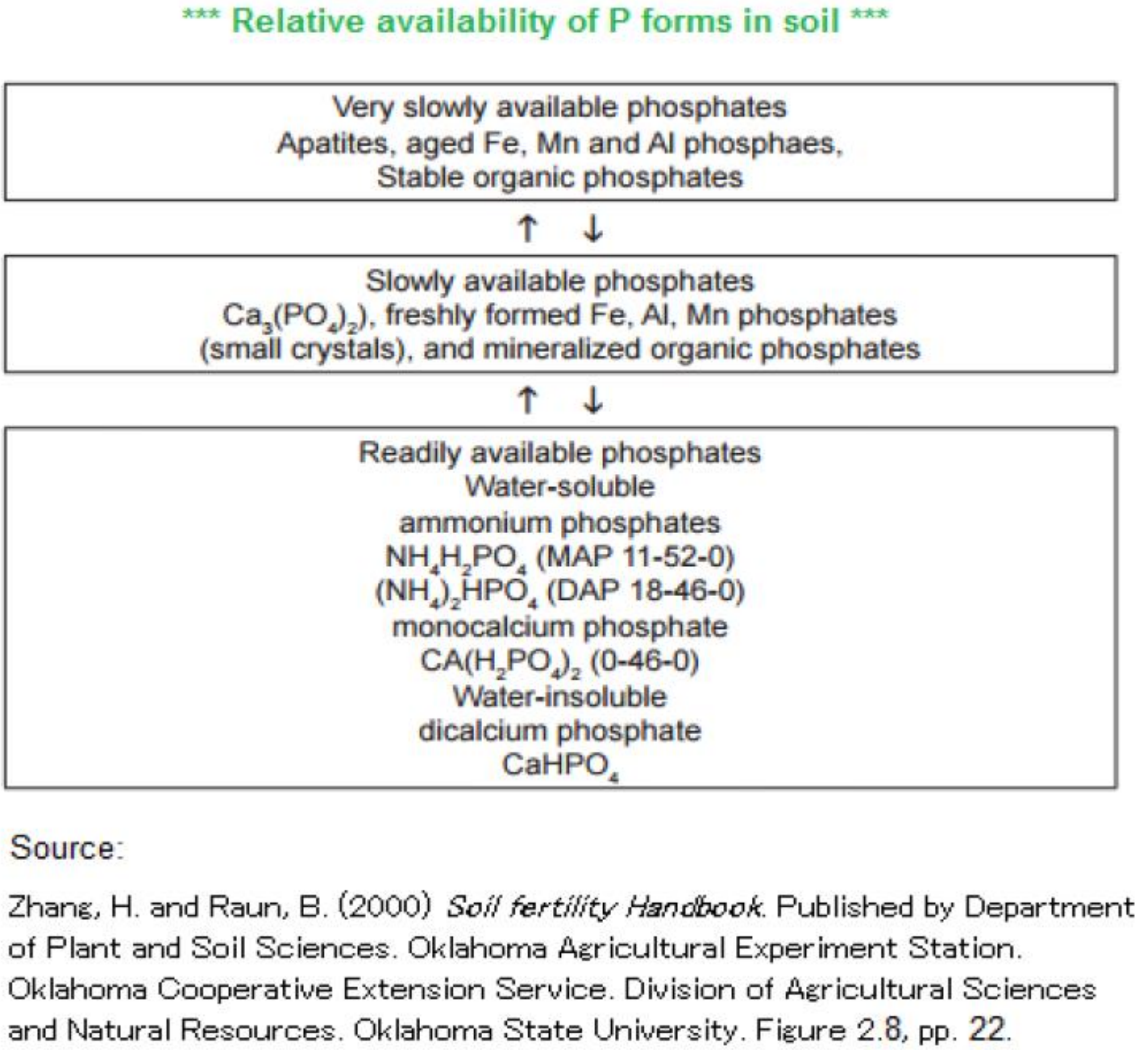


Figure 2.3. Relative availability of P forms in soil.

As with N, organic matter and microbial activities influence P availability in soil, albeit in a different way. The higher the microbial activity (and subsequently the higher depletion of soil carbon), the more that P becomes temporarily tied up in inorganic P compounds (Kauwenbergh, 2010). Because P is so immobile in soil, direct contact with P is necessary before it can be taken up by living organisms (ibid.). This is where arbuscular mycorrhizal fungi become invaluable, because their narrow hyphae can reach sources of P unavailable to wider root hairs. The P taken up in this manner by the fungi is then exchanged through a mutualistic symbiotic relationship with the plant in return for carbohydrates.

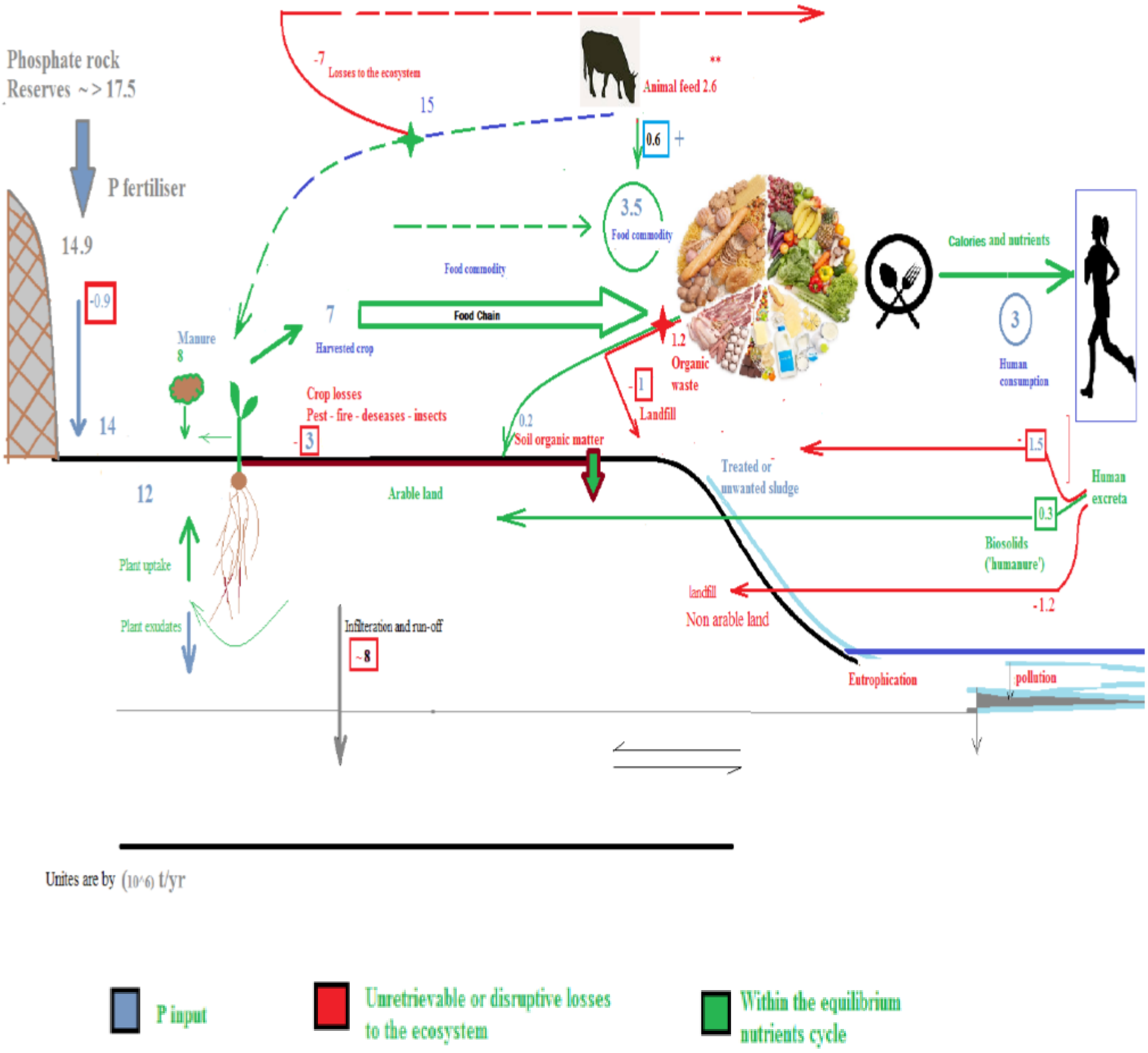


Figure 2.4. P Fertilisers budget in food production - After Cordell (2000) with slight modification based on discussion in this study.

*According to FAO, food loss/waste is roughly 30% for cereals; 40-50% for root crops, vegetables and fruits; 20% for oil seeds, meat and dairy.

** There is additional 12.1 t/yr of P input from grazing and another 0.9 t/yr coming from mining P as livestock feed supplement.

Kauwenbergh (2010) reviewed a decade of literature reporting serious concern over P scarcity, but made the point that the list of P reserves on which those reports were based (see Cordell, 2010), were not exhausive and that such fears may therefore not have been well grounded. The need to reduce consumption of inorganic P, however, and increase its efficiency

(Figure 2.4) remains an urgent requirement for sustainability of the agricultural system and healthy ecosystems in general as P is a major cause of eutrophication.

2.2. Carbon

Carbon comprises only about 0.025 % of the earth's crust, yet it is an essential component of all organic compounds (Encyclopaedia Britannica). This makes it one of the most common chemically active elements in the universe. It is additionally the dominant constituent of all living organisms, forming about 45-50% of their dry weight (Frieden, Earl, 1972; Smil, 2011).

Soil carbon, often referred to as soil organic matter (SOM), is the fraction of soil which consists of residues of animals, fungi, plants and other organisms in various stages of decomposition (Fenton et al., 2008). The more stable form of SOM is referred to as humus. Both stable and less stable organic matter are important to soil. The former provides nutrients for plants uptake and the latter stability to soil structure enabling biochemical functions such as aggregation and cation exchange (Black, 1975; Oades, 1984). Stabilisation of SOM is important for soil productivity and sustainability in addition to its significance for air quality (see carbon cycle, Figure 2.5).

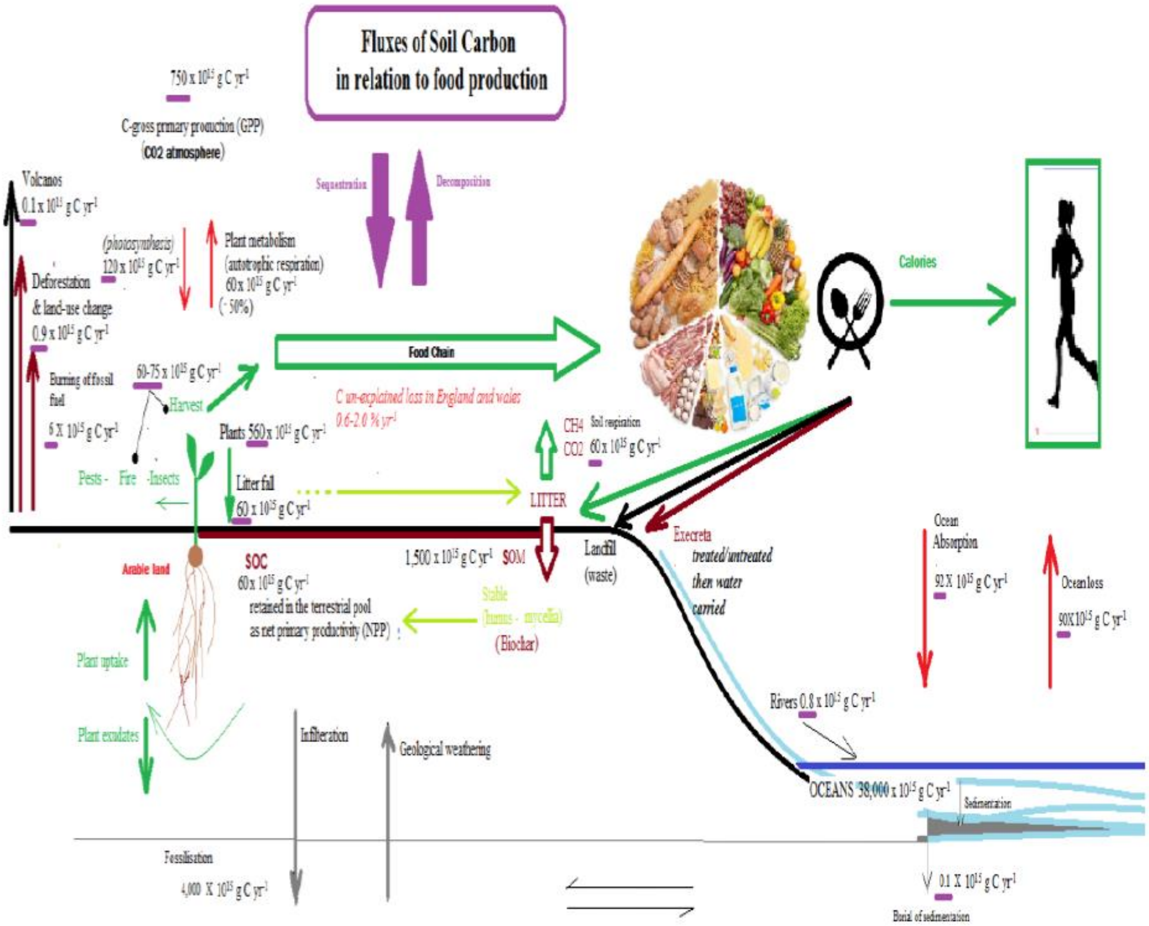


Figure 2.5. Carbon budget in food production.

Figure 2.5. - Created for this study by merging data and illustrations from Dawson, J. and Smith, P.; Schlesinger, W and Andrews, J. 2000.

2.3. The C-N nexus and nutrient flow in soil There have been conflicting results on the influence of adding nitrogen to soil in relation to carbon sequestration. Nitrogen was believed to stabilise organic matter, but this has recently been rigorously debated (Khan, 2007), as nitrogen is likely to expedite breakdown of SOM by providing energy to flourishing microorganisms (Figure 2.6). In short, when nitrogen is in excess, carbon degradation is accelerated, and the soil becomes in greater need first of organic matter for stabilisation, then of more nitrogen for equilibrium, all in a closed signalling loop.

The carbon-nitrogen budget in soil also influences availability of phosphorus (Kauwenbergh, 2010), with knock-on effects for symbiotic associations between arbuscular mycorrhizal fungi and plant roots, as will be discussed further in Chapter 4. The fact that disregard of the soil carbon-nitrogen budget and equilibrium, has actually caused seemingly conflicting results among scholars - in relation to the performance of earthworms and arbuscular mycorrhizal fungi in soil, is highlighted in Chapters 4 and 8.

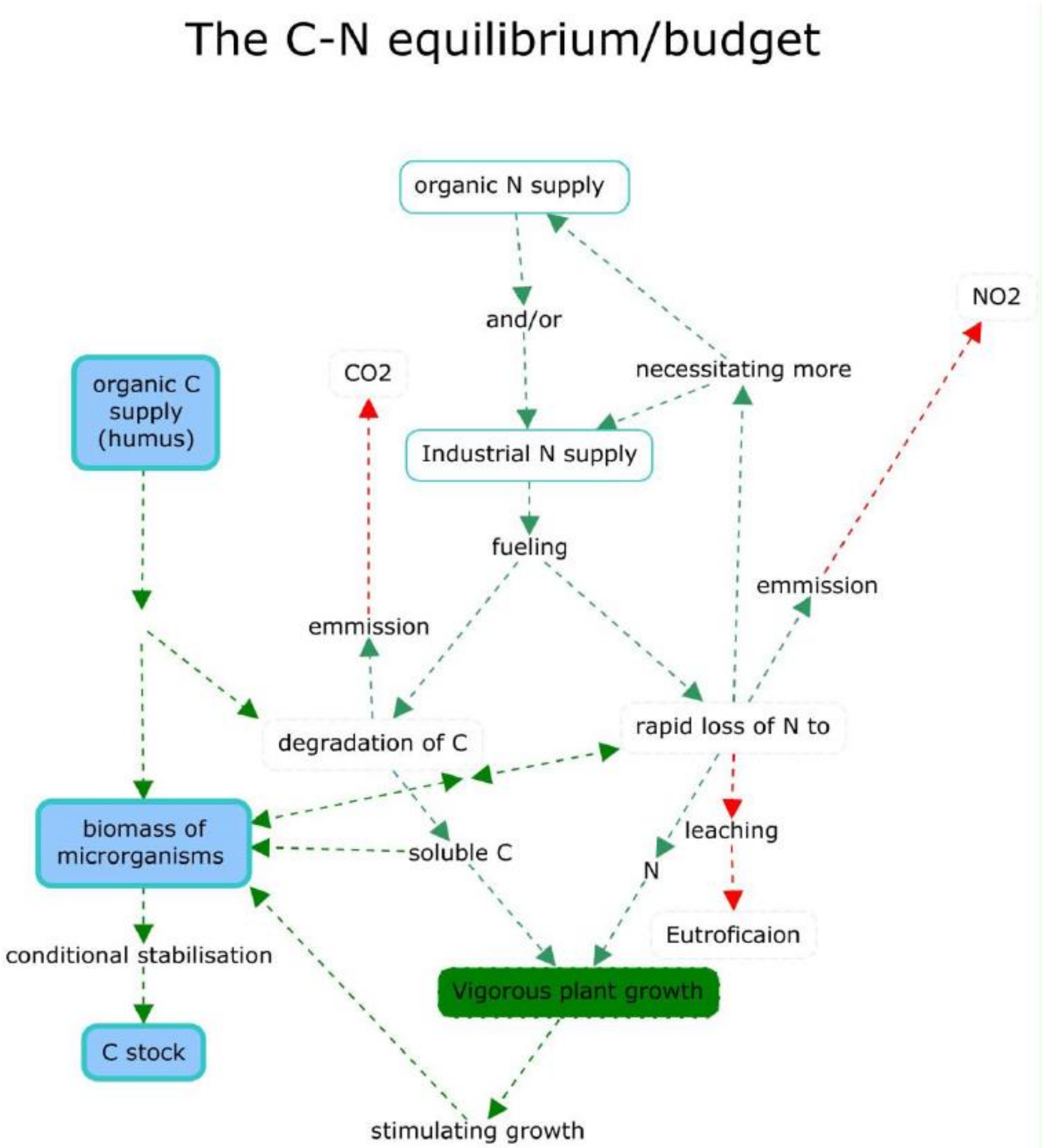


Figure 2.6. The C-N equilibrium/budget (Figures exclude animal feed) (McLaren & Skujins, 1971; Khan et al., 2007; Font & Six, 2010)

CHAPTER 3

Fungi, bacteria and the rhizosphere

Fungi and bacteria, commonly referred to as soil microorganisms, play a fundamental role in the formation and structure of all soils (Figure 3.1), and without them no soil can form (Jenny, 1994). It has been estimated that one hectare of high-quality soil contains an average of 3000 kg of bacteria and 4000 kg of fungi (Pimentel et al. 1992). While the role of bacteria in soil is widely acknowledged and has been extensively studied, that of fungi has received less attention, perhaps because they are still often mistakenly treated as a part of botany rather than as the separate biological kingdom they truly represent. It is interesting, in this context, that the biggest living organism on earth is thought to be a fungus (Schmitt, 2008).

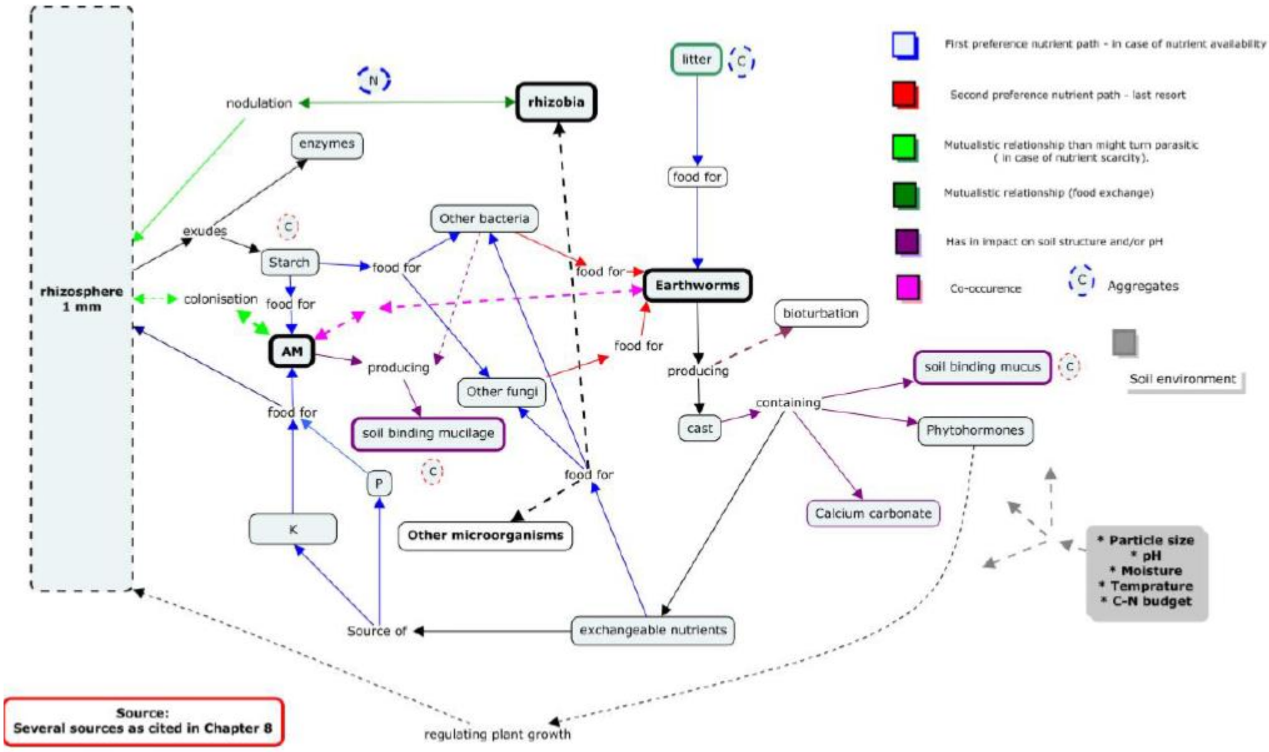


Figure 3.1. Some soil dynamics - Illustration created for this study to consolidate discussion from Chapter 8

Evidence exists that fungi predated terrestrial vegetation on earth, emerging about 460,000,000 years ago (Ainsworth et al., 2010) in association with algae in a life form known as lichens. Fungi, as described by Jenkins (2005), are key players in soil water dynamics, flow of soil nutrients and suppression of diseases. Fungi are enormously important as crop pathogens, with about 40% of an annual total of $550 billion of global crop loss being caused by plant diseases. Their ability to suppress disease is less publicised, but many fungal species carry out this role either by antagonistic activities or by simple competition with the pathogenic bacteria and fungi species. Members of the genus *Trichoderma* provide good examples of this (Vermaa et al., 2007). Cordier et al. (1996) provide a list of some pathogenic fungi and their antagonistic counterparts, but an exhaustive list cannot yet be made since only 5% of the overall 1.5 million species estimated to exist, have been discovered and described (Hawksworth,1997).

Fungi and bacteria are jointly responsible for the decomposition of organic matter, thereby completing the nutrient cycle. Decomposition of organic matter is a subject of enormous importance because the total exchange capacity of the soil top layer of minerals (25-90%) depends on organic matter and its humic acid content (MacLaren & Skujins, 1971). Fungal hyphae and bacterial cells also excrete mucilaginous material which binds soil particles and enhances soil moisture-retention. Furthermore, through their mutualistic symbiosis with over 90% of plant species, the hyphae of mycorrhizal fungi provide an enormous extension of the root system, taking up, transporting and then trading hard-to-reach nutrients such as phosphorus and potassium in return for 5-20% of the carbohydrates generated by the plant through photosynthesis (Hobbie & Hobbie, 2006; Pearson & Jakobsen, 1993).

The ecosystem services provided by bacteria and fungi are often taken for granted (Gianinazzi, 2010), and policies which do not take them into account can result in disruption of ecosystems and their natural cycles, and can cause land degradation and pollution. A thorough peer-reviewed study by Pimentel et al. (2007) (a brief is published on Cornell University website and Science Daily) shows that about 40% of deaths worldwide result from pollution of air, water and soil, and that at a global level such pollution coupled with population growth is a key contributor to disease increase. At the same time, the greater degradation resulting from such policies means that the agriculture system, albeit currently highly productive, is unsustainable in the long run and may not be able to feed the world's growing population.

The symbiosis between rhizobial bacteria, which are beneficial Plant Growth-Promoting Bacteria (PGPB), and plant roots, especially of legumes, in the formation and development of nitrogen-fixing root nodules, has been well explored (Kinkema et al., 2006; McNear, 2013). Less attention has been paid to the role of mycorrhizal fungi in plant health and soil fertility (see Antunes, 2005), and the possibility of manipulating them for the further benefit of plant and soil remains little explored.

It has been suggested that legume roots form a 'tripartite symbioses' with both rhizobial bacteria such as species of *Bradyrhizobium*, and arbuscular mycorrhizal fungi, by releasing the same special signal molecules to the surrounding soil environment, thereby allowing inoculation and nodulation to occur (Antunes, 2005; Raman & Selvaraj, 2005). Such partnerships have been found to benefit the plant, the soil and the environment - in some cases even under adverse conditions such as medium salinity (Ebrahim, 2014).

To sustain the agricultural system so that it can feed the growing human population while decreasing pollution, degradation and disease, the current agricultural approach comprising extensive use of synthetic fertilisers, insecticides, herbicides and fungicides needs to be critically re-appraised, while the integrated deployment of biocontrol, bioremediation, and biofertilisation (Glick, 2012); is becoming a matter of global urgency. In this context, it has been found that, in comparison with the arbitrary application of chemical fungicides, the benefits of healthy dynamic interaction between fungi and the rhizosphere resulting from sustainable soil-management techniques and biocontrol tactics outweigh/neutralise the damage caused by a few parasitic fungal species (Broadbent et al., 1971; Ainsworth et al., edited by Boddy, 2010; Gomathi & Ambikapathy, 2011).

CHAPTER 4

Interlinkages between mycorrhizal fungi and certain significant constituents of arable soil

4.1. Mycorrhizal fungi and phosphorus efficiency

Phosphorus availability is critical to plant growth and prime yield (Grant et al. 2001). Scarcity at early stages of plant growth often limits production. As a result, P fertilisers are often applied prior to sowing to ensure a sufficient supply. A certain level of P deficiency, however, is required for establishing the fungal/plant arbuscular mycorrhizal association. A comprehensive review of relevant literature (Grant et al., 2011) showed that there are threshold levels of P availability above which the arbuscular mycorrhizal association declines (Valentine et al. 2001; Liu et al. 2000; Lambers et al. 2003 cited in Grant et al., 2011), and below which the arbuscular mycorrhizal association (even in abundance) would not be beneficial for the plant owing to the prohibitive scarcity of the nutrient (Ryan and Ash, 1999:2003 cited in Grant el al, 2001). The review also showed that phosphate starvation may even inhibit such fungal colonisation due to the competition for other nutrients. Under those conditions, addition of even a small amount of P can improve colonisation levels.

P efficiency resulting from low P input and arbuscular mycorrhizal association is best attainable with an ample suppy of other nutrients, particularly as N and K. Grant et al. (2011) cited several experiments by Plenchette and Corpron (1987) and Valentine et al. (2001) showing that an adequate supply of other nutrients is required for successful association.

4.2. Potential mechanism for P uptake efficiency through deployment of mycorrhizal fungi

There are several potential mechanisms by which P deficiency may affect arbuscular mycorrhizal fungal colonisation (Grant et al., 2004):

- High levels of P in plant tissues make plants less susceptible to colonization, reducing the production of fungal spores (De Miranda and Harris 1994) and of secondary external hyphae (Bruce et al. 1994).
- When P is abundant in the rhizosphere, the plant has less need for colonization, and secretion of a special signal molecule diminishes with a consequent reduction in mycorrhizal association and hyphal extention (Nagahashi et al. 1996; Nagahashi & Douds 2000).
- Phosphorus abundance in the rhizosphere may also affect membrane phospholipids, reducing permeability of the membrane realeasing carbohydrates (Graham et al. 1981). Low P concentration in soil encourages carbohydrate exudation which, in turn, sustains the fungi partner, which in turn enhances P uptake.

4.3. Mycorrhizal fungi and soil carbon

One of the most important ways to mitigate anthropogenic emissions of CO_2 is terrestrial carbon sequestration. There are, however, many challenges to manipulating soil biota for carbon sequestration, including the difficulty of identifying the specific microbes and mechanisms responsible for carbon to be sequestered, particularly specially over extended spatial and temporal scales. Every fertiliser or fertliser-stimulant, including wormcasts, is likely to increase energy dissipated from soil in the form of reduced N and C due to their increased availability for plant uptake, reduction and microbial consumption. High CO_2 production is an indicator of 'vigorous mineralisation and immobilisation' regardless of the net changes in the inorganic nitrogen content or other constituents (McLaren & Skujins, 1971).

An exception may be the case of arbuscular mycorrhizal associations, which are capable of holding up to 50-70% of the total C stored in litter and soil within their physical web (Staddon et al., 2003) while increasing plant uptake and nutrient bioavailabity by their biochemical activities and the stimulation of cation exchange. Clay and humus (comprising oil stored carbon) have a higher cation exchange capacity compared to other soil constituents (Black, 1975; Oades, 1984). Mycorrhizal fungi furthermore obtain carbon energy from associated plants and do not compete with other soil saprobes for organic carbon (Olsson, 1999), exploiting the success of plants (to which they have contributed) to supply organic carbon for subsequent use (parallel to the effect of returning plant residue to soil in no-tillage land management).

There is also a growing recognition that fungi, bacteria and other microbes produce proteins and acids, such as glomalin which enhance soil aggregation by limiting degradation of organic matter (Rillig, 2004), and more research and experiments are needed in this area (King, 2010).

CHAPTER 5

Earthworms, mycorrhizal fungi and soil

"It may be doubted whether there are many other animals which have played so important a part in the history of the world, as have these lowly organized creature." Charles Darwin (1881).

5.1. Ecosystem engineers

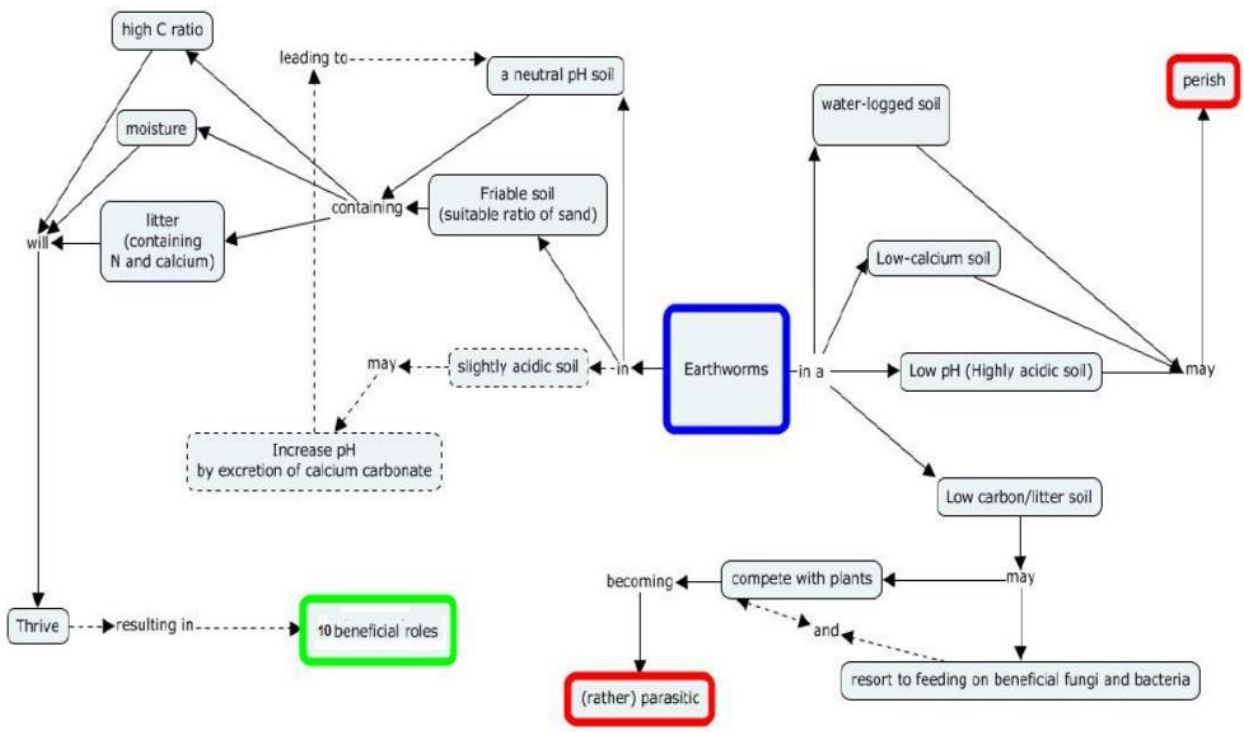


Figure 5.1. Some soil environmental factors affecting earthworms (Illustration created for this study)

Earthworms have been often described as 'ecosystem engineers' owing to the significant effect of their burrowing, secretion and casting activities on the physical structure of soil (Edwards & Bohlen 1996; Lavelle et al., 1997). They are thus also a widely accepted indicator for soil fertility (Figure 5.1). One hectare of high-quality soil contains an average of 1300 kg of earthworms (Pimentel et al., 1997). Dependent on species, nutrients and organic matter availability

(Cluzeau, 1998), in addition to influencing soil pH levels and land management strategies, earthworms may either play a parasitic or an overwhelmingly beneficial role for soil and plant growth (Edwards and Bohlen 1996) through their vertical and horizontal burrowing activities and/or casting. In this context, it is not possible meaningfully to discuss manipulation of the rhizosphere using mycorrhizal fungi and fertilisers without allocating considerable attention to the role of soil earthworms. Earthworms have often been found to play many different simultaneous roles in soil fertility. The main ones are listed below:

- Rapid and efficient biodegradation, decomposition and humification of soil litter and other organic matter (Buchanan et al., 1988 cited in Tripathi, 2004, and Suthar, 2009). Humification as defined by McLaren and Skujins (1971) is the 'general process by which plant debris is converted in the soil to humic acid'. Humic acid is defined as 'a complex of polycyclic aromatic “core” which is attached to polysaccharides, proteins, relatively simple phenols and metals' (McLaren and Skujins ,1971, pp. 45, 50). Earthworms comminute 90-100% of surface litter (Raw, 1962; Knollenberg et al., 1985), incorporating it into the deep mineral soil (Barois et al., 1987; Edwards & Bohlen 1996). In doing so, they maximise its surface area and subsequently its exposure to microbial attack (Seeber et al., 2008). Microbial biomass and activity have been generally found greater in earthworm casts than in surrounding soil (Haynes & Fraser 1998; Scullion et al., 2002 cited in Cluzeau et al., 1998). Because of such ability to expedite biodegradation of organic compounds through stimulating microbial activity and enhancing soil aeration (Cluzeau et al., 1998), earthworms have been deployed for land-pollution bio-remediation. As they may additionally neutralise contaminants by feeding on them, earthworms are strong agents of land restoration. Cluzeau et al. (1998) showed a 36% increased degradation of organic compounds in the presence of earthworms.

• Biocontrol of many pests and parasites (Blouin et al., 2005). The defence mechanisms of earthworms can benefic plants by repelling many pests and parasites. Edward & Buhlen (1996) point out that the coelomic fluid of *Eisenia fetida* smells of garlic (hence the name 'fetida'). Coelomic fluid contains many different crystals in suspension, including calcium carbonate and mucocytes which gives the fluid its mucilaginous (sticky) attribute. Edward & Buhlen (1996) stated that in stressful situations many earthworms secrete such fluid through dorsal pores as a protection from predators and/or to prevent dessication.

• Increasing soil porosity to enhance water infiltration and drainage (Shipitalo et al., 2004) (Figure 5.2).

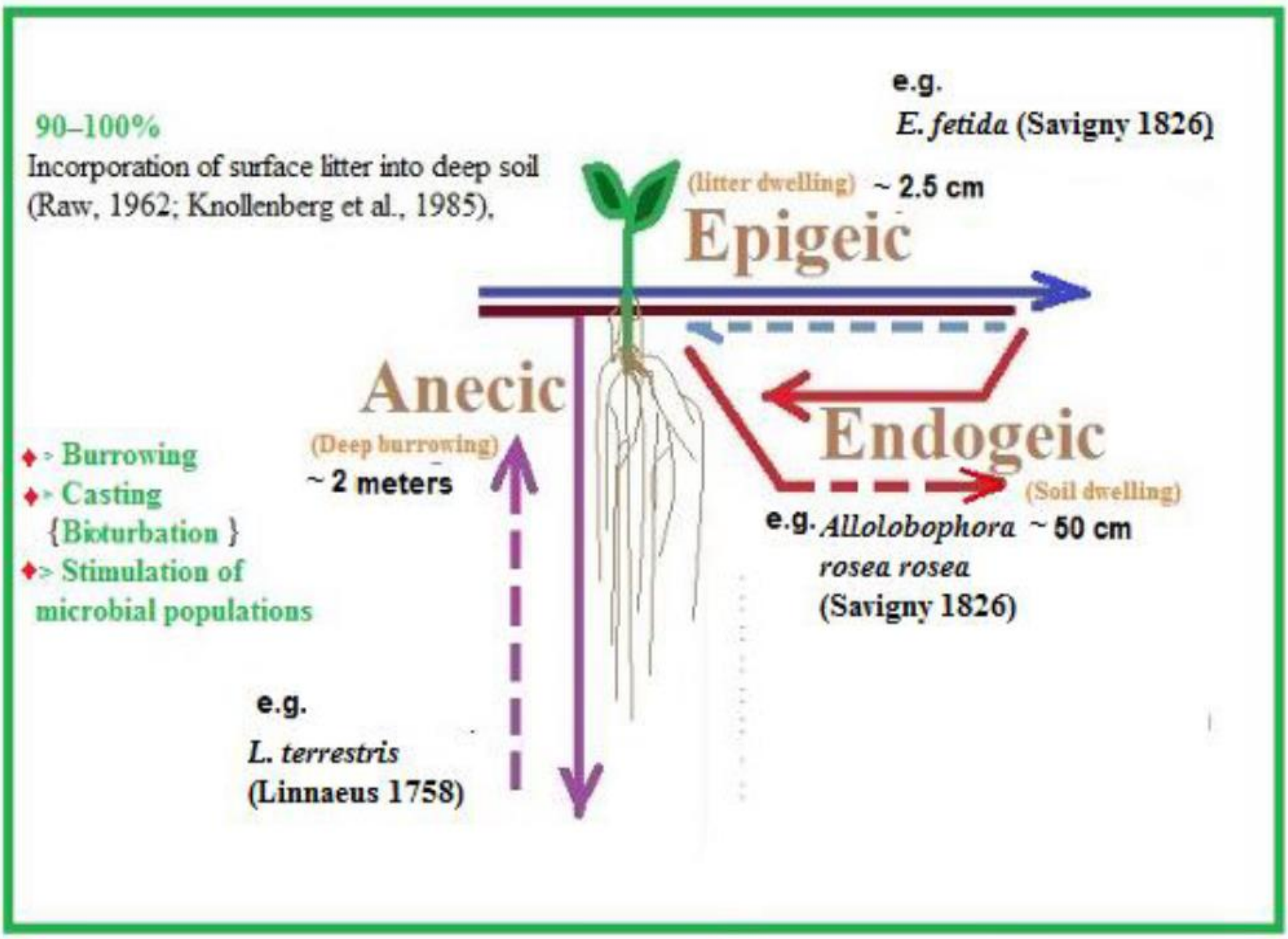


Illustration 1: Figure 5.2. Types of earthworms and their main actions in soil (created for this study from several sources).

- Enhancing soil aggregation and moisture-holding capacity by secretion of mucus and calcium carbonate (Edward & Buhlen, 1996). Earthworms feed on litter containing calcium, processing it into calcium carbonate (Darwin, 1881; Robertson, 1975). Soil rich in calcium carbonate is typically richer in organic matter (carbon) as calcium coagulates humus colloids, protecting them from dispersion and subsequent eradication (Jenny, 1994). Through a double loop process also known as bioturbation, earthworms increase soil porosity while mixing its components and enhancing its aggregates (Knollenberg et al., 1985; Blenchart et al., 1997; Edwards & Bohlen 1996; Lavelle et al., 1997). Earthworms bring 10-500 t/ha/yr of soil to the surface (Pimentel et al., 1997) and may ingest as much as 500 t/ha/yr of soil thereby churning and blending the soil (Pimentel et al., 1997). Bioturbation restructures soil particles, enhancing its hydraulic cycles, oxygen availability, aggregate, and mineral fertility.

- Antibacterial characteristics. The coelomic (body) fluid and cocoon albumen of the earthworm *Eisenia fetida* have been shown to possess antibacterial qualities. The 'bacteriostatic effect' operates only against highly pathogenic soil bacteria (Lassègues et al., 1989; Valembois el al., 1982; Bruhn et al., 2006; Bilej et al., 2010). The body fluid of *Eisenia fetida* has also been found to naturally inhibit in vitro growth of two worm pathogen bacteria (*Aeromonas hydrophila* and *Bacillus megaterium*) (Lassègues et al., 1989).

- Stimulation of (bacterial) production of plant-growth phytohormons. Interaction between earthworms and other soil biota results in production of important plant growth hormones (auxins, phytohormones and humic acids) which together act as plant regulators (Blouin, 2005; Arancon, 2006), enhancing plant growth in 80% of cases (see Scheu, 2003; Phuong et al., 2011; Lubbers, 2013; Atiyeh et al., 2002; Arancon , 2006; Sinha, 2009; Puga-Freitas et al., 2012) and increasing soil fertility as compared to conventional compost (Chaoi, 2003). Such plant growth regulators are probably produced by microorganisms in the presence of earthworms, as indicated by a concomitant 46% increase in IAA production by cultivable bacteria (Puga-Freitas et al., 2012a). Overall results suggest that adding 20–40% by volume of vermicompost to plant pots increases plant production to a maximal value compared to other organic or inorganic fertilisers (Atiyeh et al., 2000).

- Feeding selectively on fungi, bacteria and protozoa (preferring anaerobic, often pathogenic, species). Earthworms have been found to feed preferentially on first metabolites as fresh litter, while second metabolite food such as fungi, bacteria and protozoa are used by earthworms as a secondary food resource. Passage through the earthworm gut decreases the total soil microbial biomass and increases the active components of microbial biomass (Zhang et al., 2000). Another study showed that dead organic matter and root deposits are more important for earthworm nutrition than mycelium of mycorrhizas (Bonkowski et al., 2000).

- Probable stimulation of the arbuscular mycorrhizal root symbiosis (Edward & Bohlen, 1996). Earthworms and mycorrhizal fungi often co-occur but the reason for such co-occurence is not fully understood or adequately explored (Zaller & Frank, 2011). Earthworms may help increase beneficial arbuscular mycorrhizal populations, which may in turn help improve nutrient availability to plants (Gormsen et al., 2004). Earthworms, however, may in certain conditions compete with those fungi for soil carbon and nitrogen.

A study conducted by Eisenhauer (2008) showed no evidence of interactive effects between earthworms and arbuscular mycorrhizal fungi having a significant influence on the performance of the plant studied. However, since each of them has its own separate (and significant) impact on plant growth and on each other, more studies are definitely required to examine such an integrative impact in depth (Figure 5.3), particularly because earthworms may have a profound impact on the arbuscular mycorrhizal fungal plant symbiosis through several mechanisms:

A. Directly, by for example damaging fungal hyphae, feeding on them selectively (Bonkowski et al., 2000) or dispersing their spores (Reddel & Spain, Lee et al., 1996; Gormsen et al., 2004).

B. Indirectly, by modifying nutrient availability and stimulation of other microbial activities through casting and secretion, in addition to modification of organic matter distribution into soil depth (thereby affecting the base for cation exchange). Whether colonisation of roots by mycorrhizal fungi would be reduced (Lawrence et al., 2003) or increased in the presence of earthworms seems to depend on plant species (see Tripathi & Bhardwaj, 2004) and overall nutrient availability. Earthworms have been generally found to boost root growth by structuring 'nutrient-rich patches' (their casts and burrows) thereby stimulating 'root foraging' (Hutchings et al., 2000).

C. By slightly modifying soil pH (discussed in the next Chapter). Recent experiments suggest that earthworms and arbuscular mycorrhizal fungi complement each other in sustaining plant nutrient uptake and growth (Yu et al., 2005; Ma et al., 2006 cited in Eisenhauer, 2008).

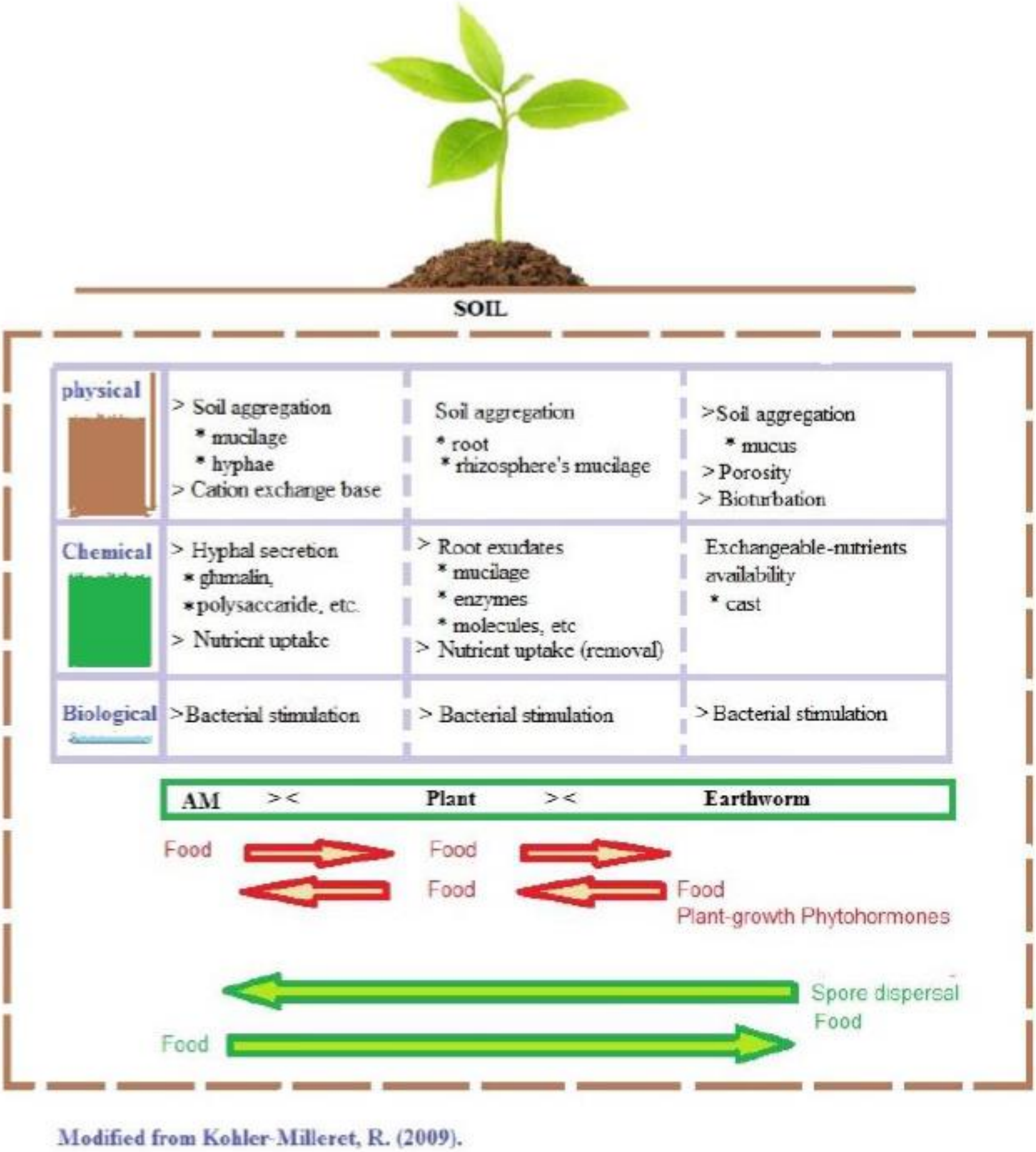


Figure 5.3. Arbuscular mycorrhizal fungi, plants, earthworms and soil, a complex and under-studied relationship. - After Kohler-Milleret, R. (2009).

- Bioliming (an often overlooked role). Soil loses its calcium carbonate over time. For example, $CaCO_3$ in a soil with original levels of 9-10% reached the value of zero after 300 years, due to leaching (Hissink, cited in Jenny, 1994). Loss of calcium carbonate transforms the physical structure of soil and increases its acidity. The common earthworm, *Lumbricus terrestris*, has been found to produce about 0.49 to 3.64 mg (individual/day) of $CaCO_3$ granules (Gago-Duport et al., 2008; Lee et al., 2008; Fraser et al., 2011; Brinza et al., 2013 cited in Versteegha et al., 2013). Other earthworm species are likely to produce similar quantities. Several studies have been conducted on the calcium carbonate produced by earthworms since Darwin (1881) first wrote about it, but they were often focused on the function of calciferous glands in the physiology of earthworms (Robertson, 1975) or the potential role of earthworms in CO_2 sequestration - since the production of $CaCO_3$ might theoretically bind carbon atoms into its crystals for as long as 300,000 years (Versteegh et al., 2013). However, $CaCO_3$, in the present context, is an efficient (moderate) phosphate binder and pH regulator. Salisbury (1924 cited in Robertson 1935) found that worm casts are generally less acid than the surrounding bulk soil. Carbonate in casts was approximately 200% that of the soil, leading to the proposal that calcareous particles from earthworm calciferous glands neutralise soil acids as well as the body fluids of the earthworms themselves as Darwin had also believed.

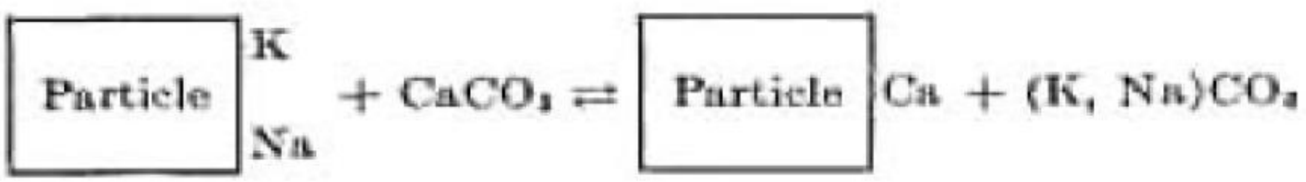


Figure 5.4. Calcium carbonate increasing availability of monovalent ions (in Jenny, 1994).

Laboratory experiments (Kay, 1929, cited in Jenny, 1994; Jenny & Shade, cited in Jenny, 1994) have shown that calcium carbonate generally enhances the exchange of monovalent ions, and thus increases their availability for plant uptake (Figure 5.4).

Calcium carbonate works as a binding agent (see Yanamadala, 2005) to phosphorus in neutral soils, according to the following reaction mechanisms (Yanamadala, 2003).

$2H_3PO_4+3CaCO_3(ag)$ --> $Ca3(PO_4)^{-2}(s) + 3CO_3^{-2} +H$ [equation 1]

$2H+CO_3^{-2}$ □ $H_2O(1)+CO_2(d)$ [equation 2]

An experiment has been conducted to test calcium carbonate as a potential phosphate binder in an effort to decrease phosphate concentration in water. The calcium carbonate was found to be highly significant ($p<0.0001$) in decreasing phosphate from an average of 1.5 ppm to 0.4 ppm, an average decrease of approximately 70%. The carbonate did also slightly raise the pH of the water (Yanamadala, 2003). In earthworm casts, phosphate does not necessarily get 'locked' in the compound, as calcium carbonate is a moderate phosphorus binder (Figure 5.5) compared with aluminium and silicon for example. Earthworms also excrete phosphatase (Krishnamoorthy, 1990).which is likely to 'unlock' phosphorus rapidly. In this case, there is a possibility that the dual action of phosphorus binding by calcium carbonate (excreted by earthworms) and the phosphorus release stimulated by phosphate-solubilizing bacteria and faecal phosphatase activity of earthworms (Satchel & Martin, 1984; Krishnamoorthy, 1990; Tripathi & Bhardwaj, 2004) in addition to the ability of arbuscular mycorrhizal fungi to acidify the rhizosphere and modify the redox potential around both mycelium and roots, as discussed earlier, regulate the release of phosphorus ions in favour of arbuscular mycorrhizal fungi colonisation requirements, while also making it more bioavailable (Lee, 1985) for plant uptake. This might hypothetically be one reason why earthworms and arbuscular mycorrhizal fungi often co-occur.

- Earthworms may also be candidate organisms to modify the interaction between ecosystems for conservation purposes, and to stabilise nutrient sequestration, particularly carbon and nitrogen through soil management practices (Tripathi & Bhardwaj, 2004; Don et al., 2008). Available data on the impact of earthworm species on GHG balance of soil are inadaquate and fragmentary. The effect of earthworms on organic matter stocks has not been tested significantly.

- Pulleman et al. (2005) shows that in temperate agro-ecosystems, endogeic species contribute to sequestration of carbon in soil by forming 'micro-aggregates', which tends to protect SOM against microbial decay. Abandoned worm burrows, however, showed a paradoxical rapid mineralisation of such carbon over 3–5 years (Don et al., 2008) This is relevant to the C-N budget discussed earlier. When earthworms colonise (or are inoculated) into a field conditions without adequate increase in SOM inputs, they tend to decrease the ratio of carbon as they use part of the carbon resources for their activity (such as production of $CaCO_3$), and to increase the ratio of reactive nitrogen (Cluzeau et al.,1998). This and other recent studies have shown that earthworms enhance stabilisation of SOM only when organic residues are returned to soil (Fonte & Six, 2010). In this context, several studies confirmed that soil organic matter levels decline after the transition to conventional arable systems with annual ploughing (Riley et al., 2008; Cluzeau et al, 1998).

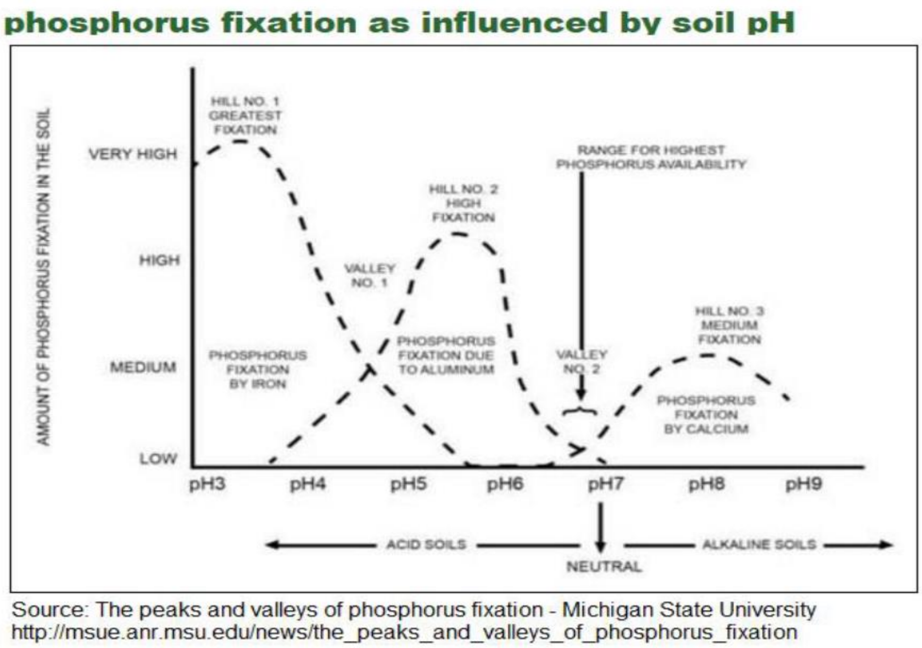


Figure 5.5. Levels of P fixation as influenced by soil pH.

Based on this, and to sum up, earthworms and arbuscular mycorrhizal fungi might co-occur for some or all of the following reasons:

1. Earthworms may increase dispersal of arbuscular mycorrhizal fungi spores.
2. Earthworms might protect arbuscular mycorrhizal fungi from antagonistic organisms and predators due to their antibacterial activities.
3. Earthworms casts might, hypothetically, be regulating phosphorus release in favour of both plants and arbuscular mycorrhizal fungi.

5.2. Vermicompost, arbuscular mycorrhizal fungi and phosphorus efficiency

Considering all such factors, vermicompost has been used in the present work for the phosphorus-efficency experiment. Depending on several factors - among which some are not yet well understood, earthworms generally help arbuscular mycorrhizal fungi to colonize, spread and flourish by dispersing their spores, increasing the dispersal of antagonistic microbes and making phoretic associations with biopesticidal soil organisms (Shapiro et al., 1993). Earthworms, however, might also in some cases, spread pathogens and weed seeds (Edwards & Bohlen 1996). To avoid such over-complexity, 2% of earthworm cast, (normally) containing random amounts of earthworms eggs was used as a general treatment to simulate the living-soil field environment, and as an NKP starter.

CHAPTER 6

P-efficiency experiment

6.1. Overall aims, specific objectives and hypothesis

6.1.1. Overall aims

The experiment examines the potential of manipulating the rhizosphere to maximise its capacity for nutritient gains, with a focus on phosphorus, to potentially reduce phosphorus losses and eutrophication, which would, in turn, have implications on food intensification and protection of the environment discussed in Chapter 1 and consolidated in Chapter 7.

6.1.2. Specific objectives

To measure the difference in biomass resulting from different treatments receiving inorganic fertilisers in different spatio-temporal settings, and the level of extractable phosphorus level in bulk soil against its availability in the rhizosphere and against the applied amount of fertilisers, among those different treatment after six weeks of being placed in a growth cabinets.

Although arbuscular mycorrhizal fungi were deployed in all the treatments, there was some potential to explore how these fungi enhance phosphorus uptake by placing inorganic phosphorus at different distances and availability levels from the growing plant root, and particularly to explore whether arbuscular mycorrhizal fungi have an affinity for phosphorus. Grant et al. (2004) have provided a list of why arbuscular mycorrhizal fungi often enhance phosphorus uptake (see the text box below). Since the other reasons in that list have already been well studied, and are thereby self evident, the only potential reason explored in the present work was whether arbuscular mycorrhizal fungi have an affinity for phosphorus.

6.1.3. **Hypothesis**

Background

Grant et al. (2004) have provided a list of why arbuscular mycorrhizal fungi often enhance phosphorus uptake:

1. Arbuscular mycorrhizal fungi *might* have an affinity to phosphorus.

2. Mycorrhizas produce phosphatases which are able to mobilise phosphorus from organic sources (Tarafdar & Marschner, 1994a, 1994b).

3. Even without phosphorus affinity, and since the radius of hyphae is much smaller than that of root hairs (~ 0.005 mm vs. 0.15 mm), phosphorus concentration in soil solution around hyphae is always higher than in the phosphorus depletion zone around roots, and this enables hyphae to absorb more phosphorus in low phosphorus soil.

4. Mycorrhizas also posses certain biochemical characteristics which differ from those of roots. Mycorrhizas can acidify the rhizosphere in a way that modifies the redox potential around both mycelium and roots, and thereby expedites release of phosphorus ions from soil to solution (Rigou & Mignard 1994; Bago & Azcon-Aguilar 1997; Hinsinger 2001 cited in Grant et al., 2004).

These different mechanisms were found to maximise response to phosphorus deficiency (Lambers et al., 2003), but not to phosphorus starvation.

Based on the discussion in (4.1) the treatment with soil P-capsule is expected to have the highest P efficiency albeit the treatment which received NPK is expected to have the highest biomass [based on the Law of the Minimum (Justus von Liebig, 1840 cited in Gorban et al., 1992]. Based on the discussion in the same section and in Chapter 4, arbuscular mycorrhizal fungi are expected to colonise the roots of all samples.

6.1.4. Research questions

- How important is a holistic approach to rhizosphere processes despite the challenges?
- What are the possible rhizosphere biochemical interactions resulting from managing the spatio-temporal aspect of fertiliser application in a way which encourages arbuscular mycorrhizal colonization?
- Is it possible to increase P efficiency by stimulating arbuscular mycorrhizal fungal colonisation and, if so, what are the potential mechanisms of phosphorus efficient uptake?

6.2. Material and methods

The experiment, based on previous scientific findings discussed in Chapter 4.1 that arbuscular mycorrhizal fungal colonization is greater in conditions of phosphorus deficiency (but not scarcity or starvation), used soil low in phosphorus and high in organic matter with the potential of facilitating arbuscular mycorrhizal fungal colonization. Such phosphorus deficient soil also allows testing the level of benefit from phosphorus application at different distances and release spans (timing).

Suitable soil, which was loamy and deficient in other nutrients, was collected from SRUC's experimental study site at Bush Estate, at the end of the 2015-2016 growing season. The soil was sieved through a -> 0.197 mm sieve; aggregates of organic matter, earthworms and pebbles were discarded. Some organic matter stayed in the soil as it went through the sieve. The soil was then distributed to a series of pots (these were recycled water bottles, described below, and are referred to in the present text as columns). Each column received a kilogram of soil and 20 grams (2%) of specially prepared vemicompost containing a blend of food waste, paper and saw dust fed to 50 g of *Eisenia fetida* over three months. No manure or urine was used in the process. Vermicompost used for soil modification was 3 months old. Vermicompost-tea used for inoculation was from 2 months old vermicompost.

6.2.1. Columns upload and labeling

Recycled water bottles were used as columns (pots).

To explore the possibility of increasing nitrogen and phosphorus uptake efficiency through stimulating inoculation of mycorrhizal fungi in a nutrient-limited environment, the following steps were carried out:

- five (X5) plant testing-pots (treatments) were prepared to be examined over 6 weeks from sowing;
- one pot received an 18g top dressing of 7-7-7 NPK prior to sowing as detailed below;

- Three other pots received 6g of P each, applied at different distances and levels of release (see table);
- Another pot received no P;
- All replicates were treated with 2% vermicompost as a potentially convenient environment for infection and as an NPK starter for the poor nutrient soil used (see Chapter 5 for further explanation). Vermicompost was prepared by adding 50g of living *Eisenia fetida* (also known as the red wiggler or tiger worm) to a 3:1 paper (dry volume)-foodwaste formula with addition of naturally occuring tannin, noting that paper loses volume when wet to approximately a 1.5:1. No manure or urine was added. Food-waste was fortified by calcium extracts (see Russell, 1961).
- All replicates were inoculated with arbuscular mycorrhizal fungi at the time of sowing.

6.2.2. Planning examination of outcome

- Shoot and root biomass were recorded as a measure of plant productivity;
- bulk soil and rhizosphere soil was collected for analysis (extractable soil phosphate, mineral N and DOC contents) to measure P mobilisation levels in the rhizosphere as compared to bulk soil before sowing and after harvest;
- microscopic examination was made of root hairs to observe evidence of root-arbuscular mycorrhizal symbiosis symbiosis, if present;
- morphological characteristics of shoots and roots were observed during harvest to record visible biochemical indicators.

6.2.3. Application of organic and inorganic fertilisers

Calculation of the optimum level of applied phosphorus fertiliser was key to the success of this experiment. According to Grant et al (2004), total soil phosphorus ranges from 100 to 2000 mg P kg–1 soil (~ 350 to 7000 kg P ha–1) concentrated in the surface 25 cm of the soil. Only a small portion of this P, however, is readily available for crop uptake (Morel, 2002). Crop removal may range from 3 to 30 kg P ha–1. The following table shows the optimum availability of exchangeable P in soil (ibid).

P level mg/kg	100mg/kg (Threshold)	2000mg/kg (high)
P amount kg P ha–1 1kg/10^4m	350kg/10^4 m^2	7000kg/10^4 m^2
g/m (0.01kg/1m)	0.35g/1 m^2	70g/100 m^2
mg/cm^2 (0.0001kg/0.01m)	0.0035 mg/cm^2	0.7mg/cm^2

Table 6.1. Optimum availability of exchangeable P in soil.

Soil tests showed extractable P at 6.5 which is Low – Medium (Table 6.2). The recommendation for such P availability - according to the fertiliser guide is: 46 lb/ha = 21kg/ha-1 = P = 6 mg/kg-1.

Synthetic NPK 7-7-7 was applied to one set of pots (treatment) with the amount of =18 gm/kg. All other sets received 6gm/kg of synthetic P each, but at different distances and release time span. Synthetic P was applied with the aid of special cartridges and the use of agar-agar.

Sodium bicarbonate phosphorus (Olsen) soil test (ppm)	**Rating**	**Phosphate (P2O5)[2] required kg/ha**	**Ammonium acetate potassium soil test (ppm)**	**Rating**	**Potash (K2O) required kg//ha**
0-3	HR	80	0-15	HR	120
4-5		90	16-30		110
6-7		50	31-45		90
8-9		40	46-60		80
10-12	MR	30	61-80	MR	60

13-15		20	81-100		40
16-30	LR	0	101-120		30
31-60	RR	0	121-150	LR	0
61+	NR³	0	151-250	PR	0
			251+	NR³	0
100kg/ha -90lb/acre					

Table 6.2 Phosphate and Potash recommendations for Soybeans Based on OMAFRA-Accredited Soil (page). **Note**. As stated in the Plant and Soil Sciences eLbraryPRO, 'The standard soil tests for available phosphorus are generally weak acid extractants which dissolve portions of the calcium, iron and aluminum phosphates in the soil. In the sodium bicarbonate test, also known as Olsen P, for high pH soils, the bicarbonate ion removes calcium from the system, solubilizing the calcium phosphates, which are measured as an "index" of available phosphorus'. (Plant & Soil Sciences eLibraryPRO).

6.2.4. Pot design, labeling and capsule design

There were several pot designs for the replicates (Table 6.3). Columns were prepared using 1.5 litre plastic water bottles to allow a simple and affordable mechanism of P application. Bottles were cut using scissors and were drilled for water drainage using an electric drill (photo). To block light and ensure optimum root-growth conditions, the bottles then were wrapped in black plastic. They were then labeled as follows:

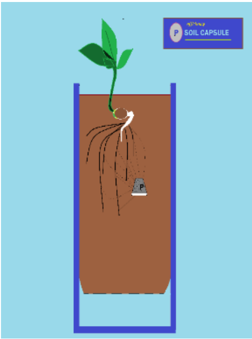
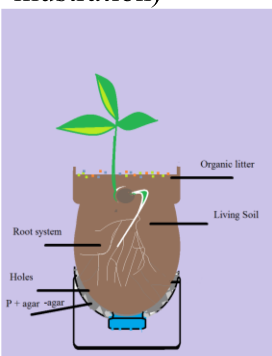
Pot design	Inorganic fertiliser in gm/kg $^{-1}$					Treatment	Analysis
Pot 1 NPK (control)	D1	D2	D3	D4	D5	AM fungi 2% vermicompost NPK 7-7-7:	Examination of plant morphological characteristics (Visual analysis of nutrient deficiencies if present). - Determine root and shoot biomass. - Examination of root, soil nutrients (phosphate, nitrate and ammonium), - AMF microscopic visualisation.
	NPK = 18	NPK =18	NPK = 18	NPK = 18	NPK = 18		
Pot 2 [Design A] (see annex a. for a full size illustration) 	A1	A 2	A 3	A 4	A 5	- AM fungi - 2% vermicompost - P Soil Capsule	
	9.17 (P=6 Agar= 0.5)	8.24 (P=6 Agar= 0.5)	8.45 (P=6 Agar= 0.5)	9.93 (P=6 Agar= 0.5)	7.40 (P=6 Agar= 0.5)		
Pot 3 [Design B]	B1	B2	B3	B4	B5	- Mycorrhizae - 2% Vermicompost - P Soil Chips	
	13.5 (P=6)	13.5 (P=6)	13.5 (P=6)	13.5 (P=6)	13.5 (P =6)		
Pot 4 [Design C] (see annex b. for a full size illustration) 	C1	C2	C3	C4	C5	- Mycorrhizae - 2% Vermicompost - P reservoir	
	(P=6) 1g agar + handful of soil	(P=6) 1g agar + handful of soil	(P=6) 1g agar + handful of soil	(P=6) 1g agar + handful of soil	(P=6) 1g agar + handful of soil		
Pot 5	N/A	N/A	N/A	N/A	N/A	- Mycorrhizae - 2% Vermicompost	

Table 6.3. Summary of the experiment framework.

Design A was the only one to use capsules. Each capsule was a plastic tube 3.5 cm long × 1 cm diam. filled with 6 g of superphosphate and 1 g of agar agar then sealed at both ends and punctured using a sewing needle to produce 100 holes penetrable by mycorrhizal fungi but not root hairs. Design B involved chips. These were small gelatinous lumps of agar agar mixed with superphosphate in roughly equal proportions. Those chips are meant to release P slowly as they need to be consumed by soil microorganisms first. Design C had a reservoir comprising 6 g of superphosphate with 1 g of agar agar wrapped around the bottle base which was punctured with holes large enough to permit access to the fertiliser by both fungal hyphae and root hairs at a certain level of growth. The reservoir was positioned to minimise possible leaching.

6.2.5. Germination, sowing and monitoring

A commercial package of *Phaseolus vulgaris* (kidney bean) seeds was purchased and the seeds allowed to germinate for three days prior sowing by placing 25 beans between moistened filter papers.

6.2.6. Timeline and sequence of experiment

Month	March/April	May				June				July				August 15th
Week	4 weeks	w1	w2	w3	w4	w1	w2	w3	w4	w1	w2	w3	w4	2 weeks
Activity														
Processing the vermicompost														
Soil collection and sieving														
Germination														
Packing columns and placement in growth cabinet														
Watering and observation														
Harvest														
Analysis														
Writing the report														

Table 6.4. Timeline and sequence of the experiment.

6.2.7. Experiment environment and dates

The experiment was conducted in a 3 × 4 m growth room equipped with cool white fluorescent lamps, model 840, Philips (mounted above a clear glass barrier, and an upward airflow distribution system using sufficient outdoor make-up air to provide ambient CO_2 levels inside the room. Room air temperature was 18°C (SD ± 2/1°C) during the light/dark period. Photosynthitically active radiation (PAR) at the top of the canopy was 400 μmol m^{-2} s^{-1} (SD ± 10 μmol m^{-2} s^{-1} while maintaining a 12-hour photoperiod. Relative humidity was 50% (SD ± 10%). Plants were placed in 1 L soil pots filled with loam soil and distributed randomly in a free drainage growth cabinet. They were watered every other day (with a two days gap over the weekend: Three times per week – Monday-Wednesday-Friday). Support sticks were erected to assist the plants to climb. Key dates for the experiments are shown in Table 6.5.

Beginning of germination	28/05
Packing columns, sowinb and application of NPK/P	01/06
Setting columns in growth cabinet	02/06
Application of support sticks	20/06
Watering	Monday – Wednesday – Friday of each week
Harvesting	19/07

Table 6.5. Experiment key dates.

6.2.8. Observations and destructive harvest

At the end of week six, the following jobs were carried out;

- observations during harvesting – max rooting depth/nodulation, plant and rooting conditions,
- weighing of harvested shoots (fresh/dry weight), splitting roots and soil depths in 2 sections (top, bottom), collecting the roots and measuring the fresh weight,
- extracting the rhizosphere and bulk soil at 2 depths (25 pots) to measure microbial biomass P, C and N,
- extracting the rhizosphere and bulk soil at 2 depths to measure extractable P, mineral N, and C,
- staining roots using stored roots,
- washing rhizosphere for dissolved soil carbon, and dissolved N and P.

6.3. Results

6.3.1. Soil properties

Determination of soil properties at the beginning of the experiment and after harvest was carried out using Skalar methods (technical note Catnr.155-006w/r issue 011609/MH/99254553). All of the soil tests were done by the John Parker, the University Laboratory's Chemist, and used the following methods. The determination of extractable PO4:P in soil was carried out using the Olson test, while the determination of N-NH4, N-NO3 and urea in soil was carried out by shaking samples with IM potassium chloride solution in a ratio of 1:10 for an hour. The extract was then obtained after filtration.

Microbial biomass P data were obtained by calculating the difference between the amounts of inorganic P (P) extracted from fresh soil fumigated with CHCL3 and the amount extracted from unfumigated soil [references for these techniques: Brookes, O.C. et al. (1982). Measurement of microbial biomass phosphorus in soil. *Soil Biol. Biochem.* **14**: 319-329. Hedley, M.J. & Stewart, J.W.B. (1982). Method to measure microbial phosphate in soils. *Soil Biol. Biochem.* **14**: 337-385]. The Microbial Biomass Carbon (MBC) data were obtained by calculating the difference in the dissolved organic matter (DOC) concentraton between fumigated and unfumigated, using this to estimate Microbial Biomass Carbon (MBC).

6.3.2. Shoot biomass, fresh and dry

Table 6.6 shows results for the biomass of fresh and dry shoots.

Treatment	A		B		C		D		E	
	shoot fresh weight	Shoot dry weight	shoot fresh weight	Shoot dry weight	shoot fresh weight	Shoot dry weight	shoot fresh weight	Shoot dry weight	shoot fresh weight	Shoot dry weight
1	17.1	2.85	14.14	1.2	12.12	2.6	1.73	1.36	9.45	1.76
2	14.6	2.81	11.61	2.37	14.41	2.55	44.25	5.45	14.21	2.33
3	15.83	1.91	14.78	2.52	15.45	2.25	48.75	5.88	16.98	2.35
4	14.95	2.2	9.5	1.78	15.11	2.42	21.87	3.48	12.56	2.36
5	14.66	2.08	16.1	2.93	13.93	2.46	35	3.91	10.5	1.7
Mean	15.428	2.37	13.226	2.16	14.204	2.456	30.32	4.016	12.74	2.1
STDEV	1.0560634	0.43261	2.645974	0.67687	1.307088	0.135388	19.00265	1.79469	3.000525	0.3386
Std error	0.4722859	0.19347	1.183316	0.3027	0.584548	0.060548	8.498243	0.80261	1.341876	0.151427

Table 6.6. *Biomass of fresh and dry shoots.*

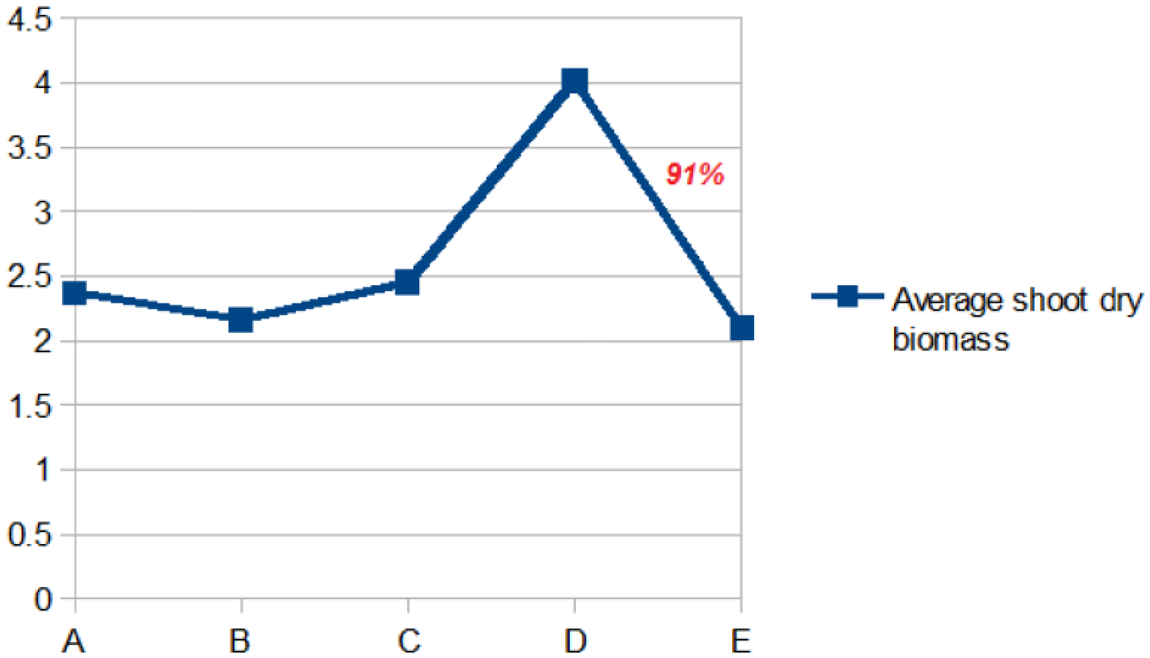


Figure 6.1. Mean shoot dry biomass in grams for each experimental pot treatment.

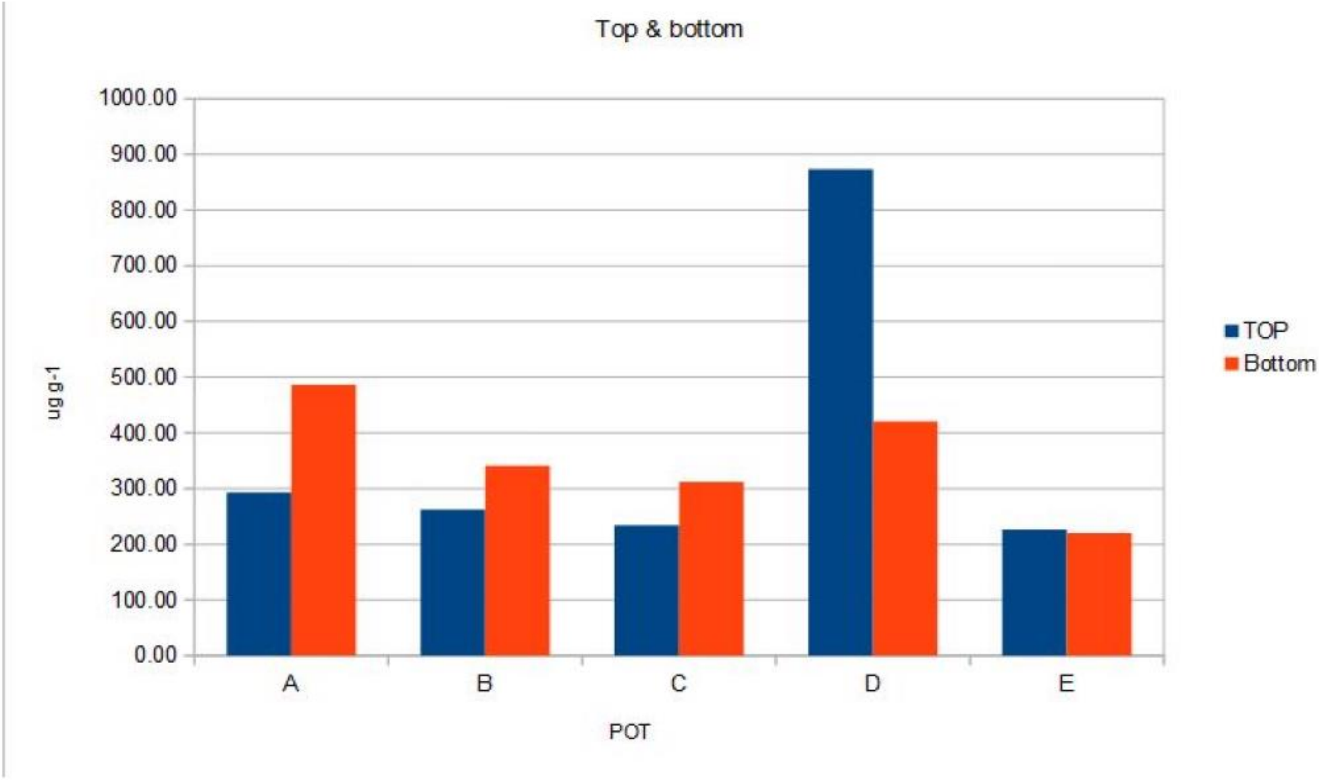


Figure 6.2. PO4:P index in bulk soil.

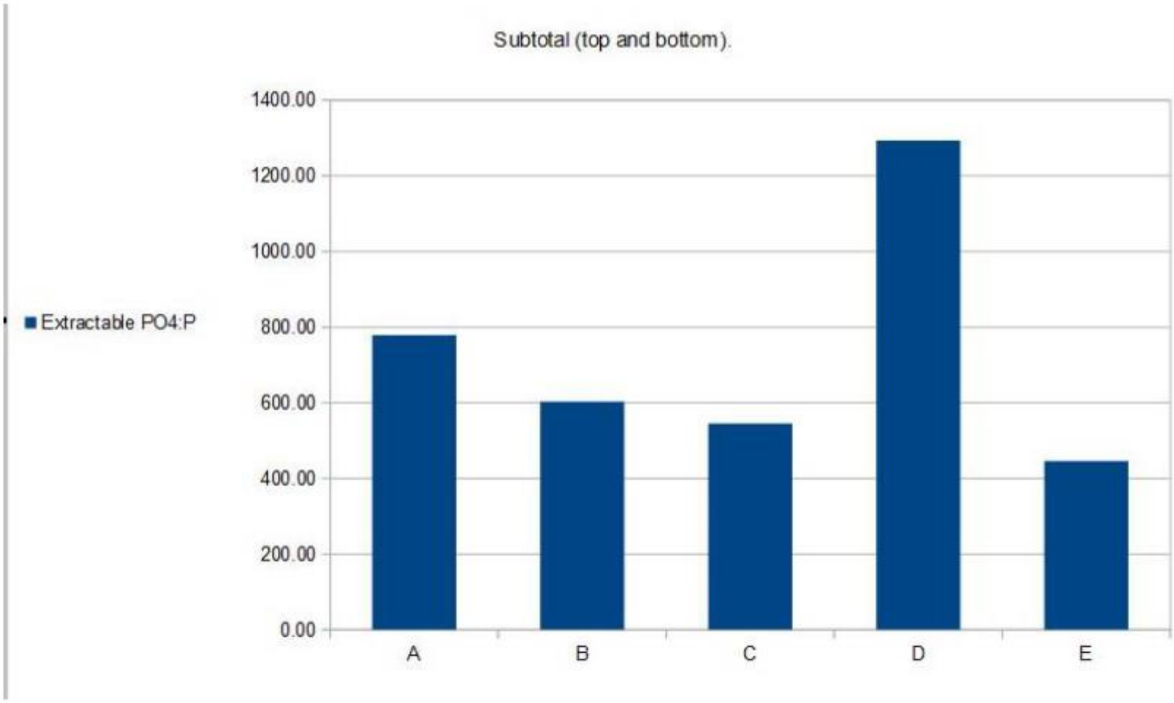


Figure 6.3. Total extractable PO_4:P in bulk soil.

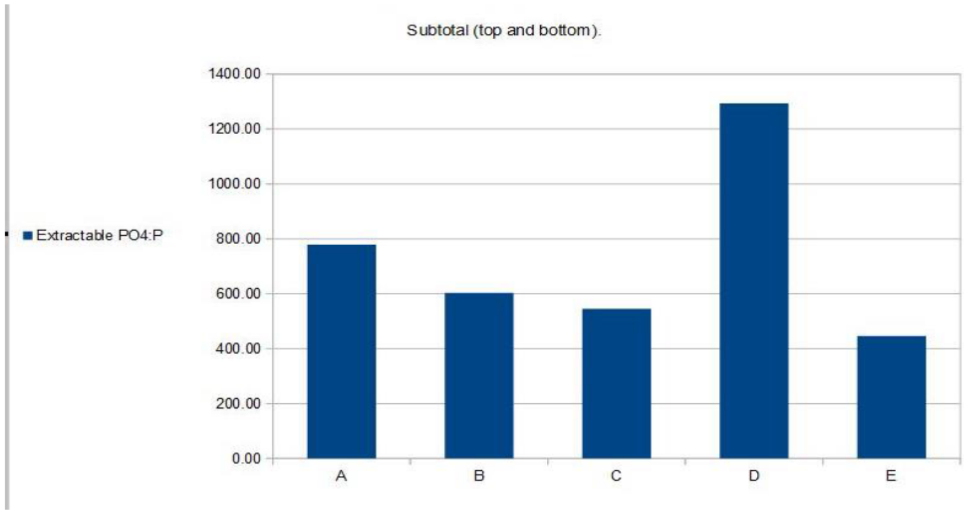


Figure 6.3. Total extractable PO_4:P in bulk soil.

PO4:P in bulk soil	
TOP & Bottom	
t at %5 calculated in Excel – Two tailed	
A/E	0.00
B/E	0.00
C/E	0.00
D/E	0.00
A/B	0.00
A/C	0.00
A/D	0.01
B/C	0.06
B/D	0.00
C/D	0.00

Table 6.7. TTEST and significance of data variability for biomass comparison.

BULK SOIL			
P-efficiency Project			
POT	Top Extractable PO4:P µg g-1 in soil DM	Bottom Extractable PO4:P µg g-1 in soil DM	Top + Bottom Extractable PO4:P µg g-1 in soil DM
A1+	346.4	498.7	845.1
A2+ 8.24	309.7	475.7	785.4
A3+ 8.45	223.8	439.9	663.7
A4+ 9.92	302.0	524.9	826.9
A5+ 7.4	282.7	492.1	774.8
Average A	292.92	486.25	779.2
B1 13.5	260.5	345.5	606.0
B2 13.5	317.6	340.3	657.9
B3 13.5	214.5	353.7	568.2
B4 13.5	260.4	314.8	575.2
B5 13.5	257.2	349.7	606.9
Average B	262.0	340.8	602.8
C1 6gm	233.6	297.3	530.9
C2 6gm	253.3	271.4	524.6
C3 6gm	247.1	277.7	524.7
CB 6gm	228.6	345.3	573.9
C5	205.8	368.3	574.1
Average C	233.66	311.98	545.6
D1 NPK	1200.5	409.4	1609.9
D2 NPK	648.9	362.5	1011.4
D3 NPK	735.0	304.4	1039.4
D4 NPK	1024.6	440.9	1465.5
D5 NPK	756.6	587.0	1343.5
Average D	873.1	420.8	1293.9
E1	240.0	224.9	464.9
E2	222.0	216.4	438.5
E3	224.5	216.0	440.5
E4	213.1	208.8	421.9
E5	231.9	234.1	465.9
Average E	226.3	220.0	446.3

Table 6.8. Data showing extractable PO_4:P in bulk soil.

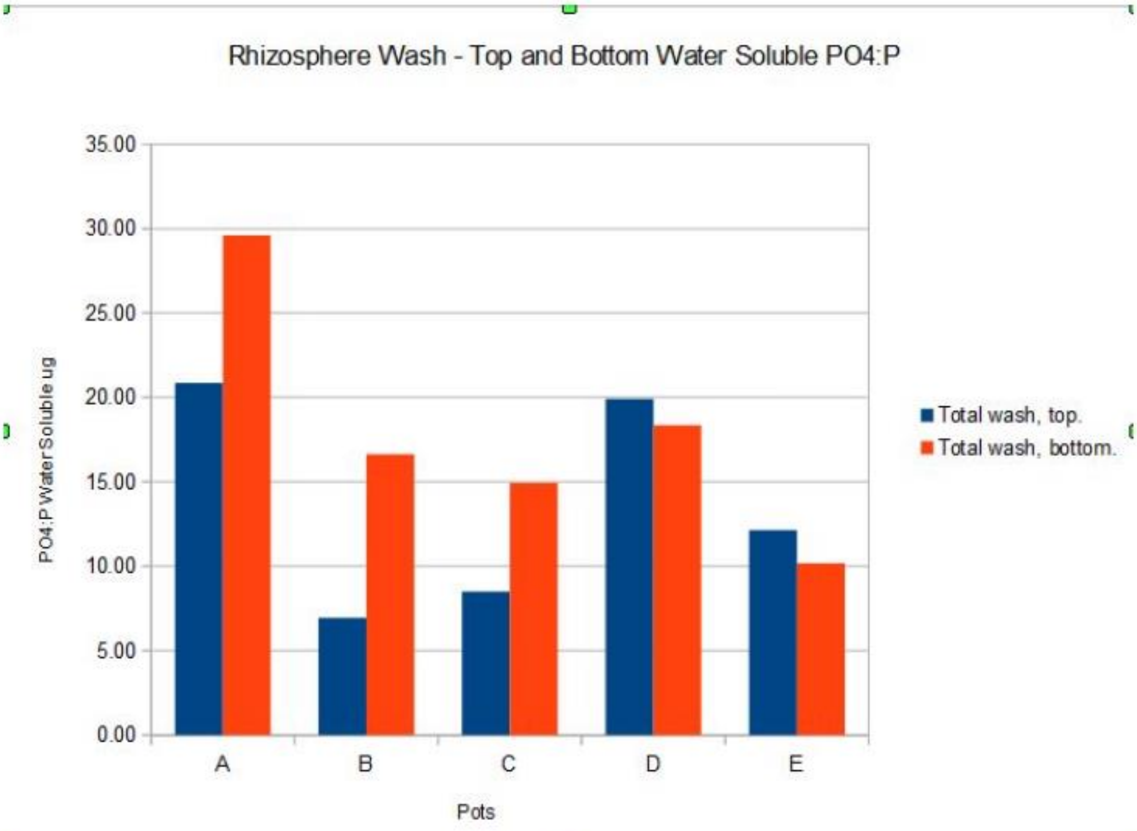


Figure 6.4. P availability in the rhizosphere.

Rhizosphere Wash PO4:P µg			
POT	Total wash, top.	Total wash, bottom.	Subtotal of Top & bottom wash
A	20.87	29.61	50.48
B	6.95	16.65	23.60
C	8.50	14.94	23.44
D	19.92	18.36	38.28
E	12.14	10.19	22.33

Table 6.9. Rhizosphere wash: top and bottom extractable PO_4:P.

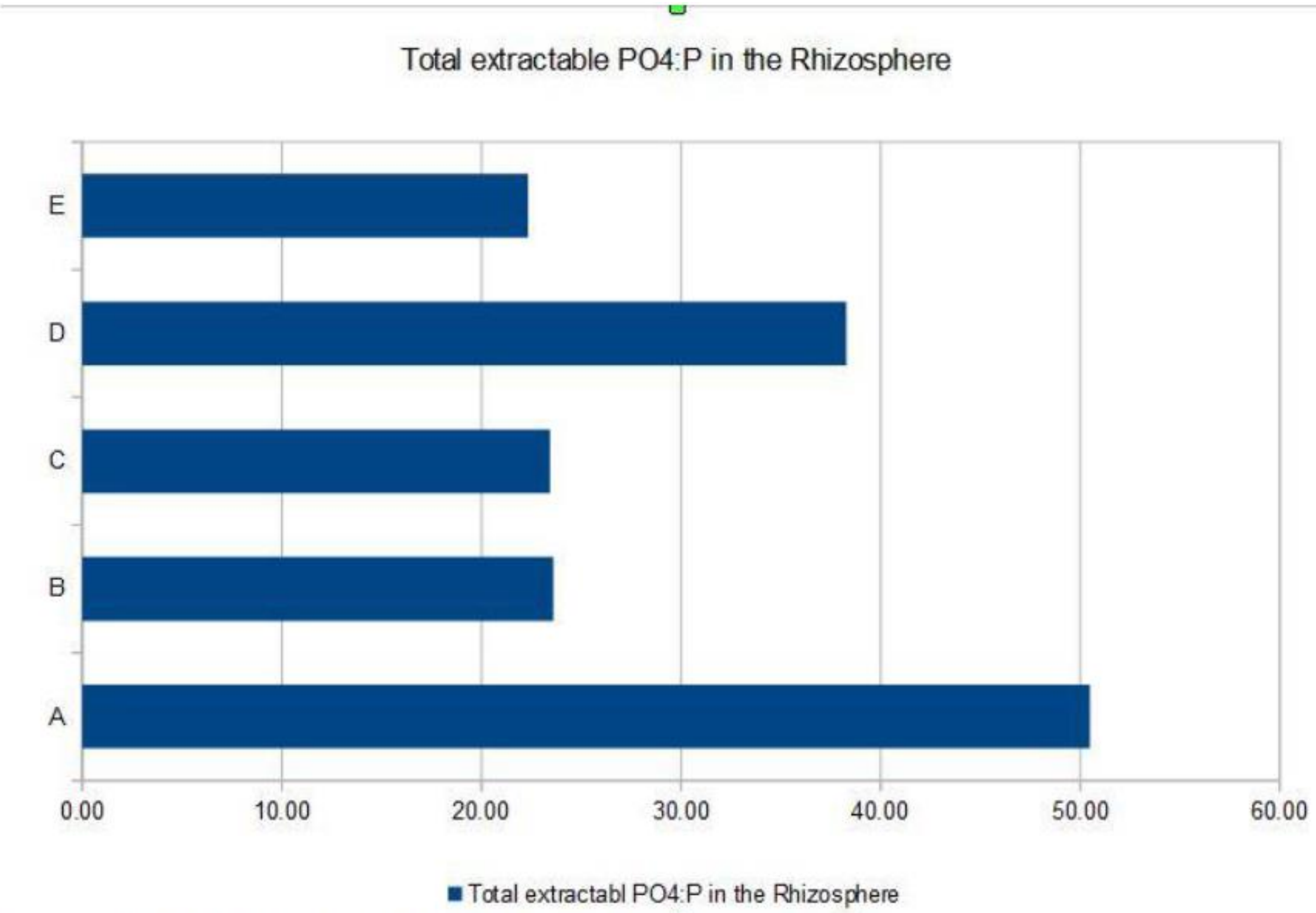


Figure 6.5. Total extractable PO_4:P in the rhizosphere.

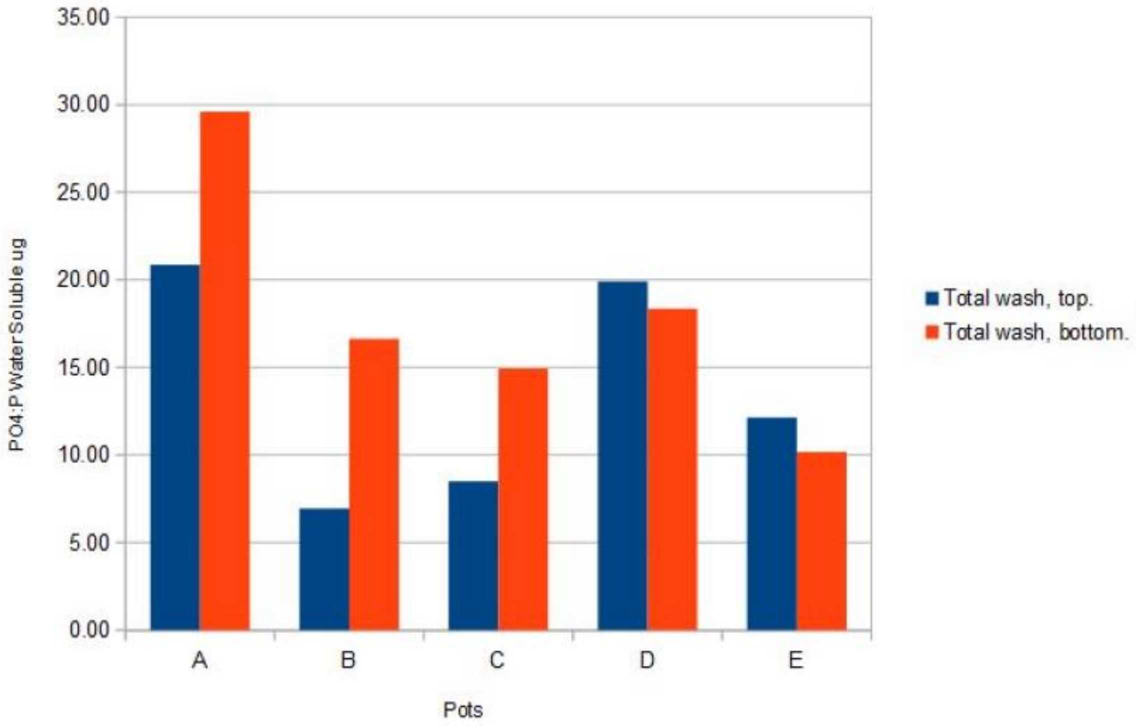


Figure 6.6. Rhizosphere wash: top and bottom extractable PO_4:P.

Figure 6.9 shows top and bottom extractable PO_4:P from the rhizosphere wash.

Rhizosphere Wash PO4:P µg			
POT	Total wash, top.	Total wash, bottom.	Subtotal of Top & bottom wash
A	20.87	29.61	50.48
B	6.95	16.65	23.60
C	8.50	14.94	23.44
D	19.92	18.36	38.28
E	12.14	10.19	22.33

Table 6.9. Rhizosphere wash: top and bottom extractable PO_4:P.

RHIZOSPHERE WASH	
PO4:P Water soluble **µg**	
TOP & Bottom (SUM)	
t at %5 calculated in Excel – two tailed	
A/E	0.17
B/E	0.94
C/E	0.95
D/E	0.45
A/B	0.04
A/C	0.04
A/D	0.45
B/C	0.98
B/D	0.33
C/D	0.32

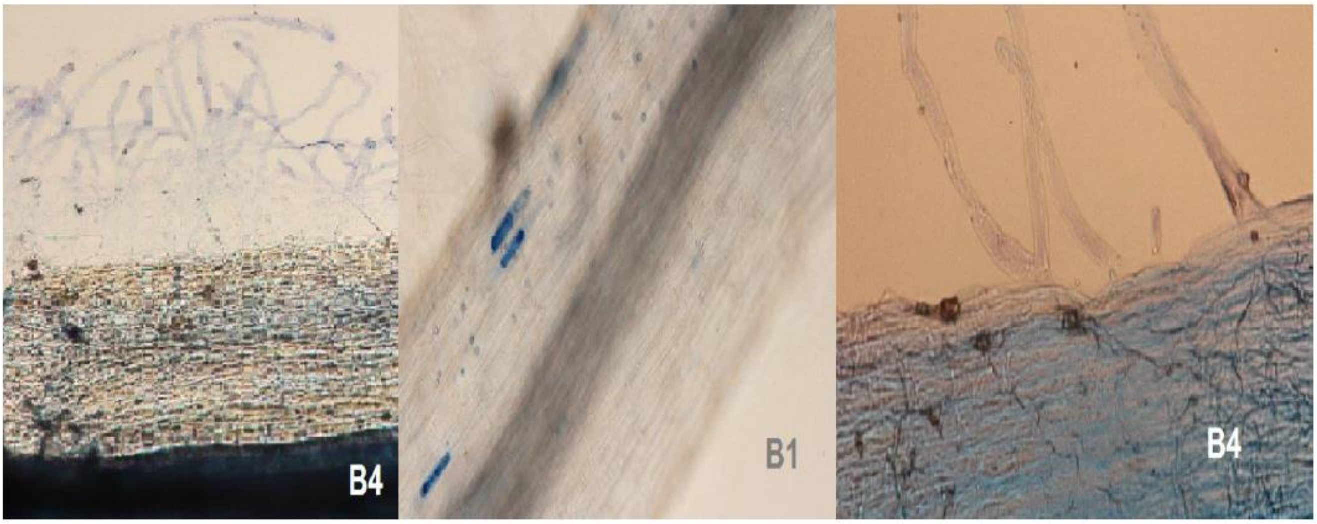
Table 6.10. TTEST results for rhizosphere wash data.

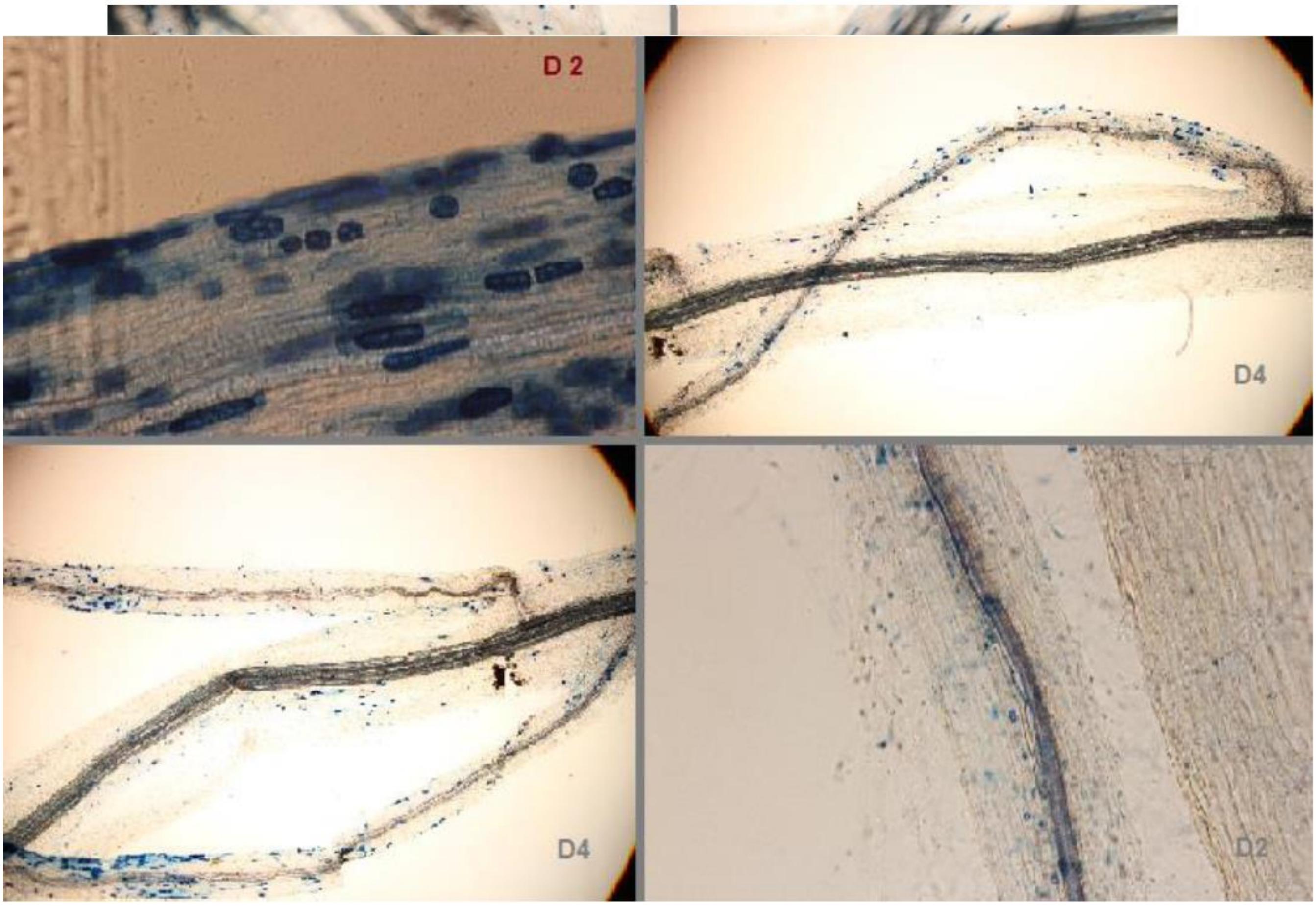
The data show that the highest level of extractable PO4:P is in the D treatment which received NPK and that was mainly at the top part of the soil. The data also show that the lowest extractable PO_4:P in bulk soil was that of E which received no P at all. Despite B and C receiving the same amount of P inorganic fertiliser, they demonstrate a lower availability of the extractable element/compound. Treatment A could not be said to have received the same amount of inorganic P because the substance was locked in the capsule which lost only 1-3 g each over the six weeks, yet, the availability of PO_4:P in treatment A is significantly higher than that of B, C and E, but is significantly lower than that of D.

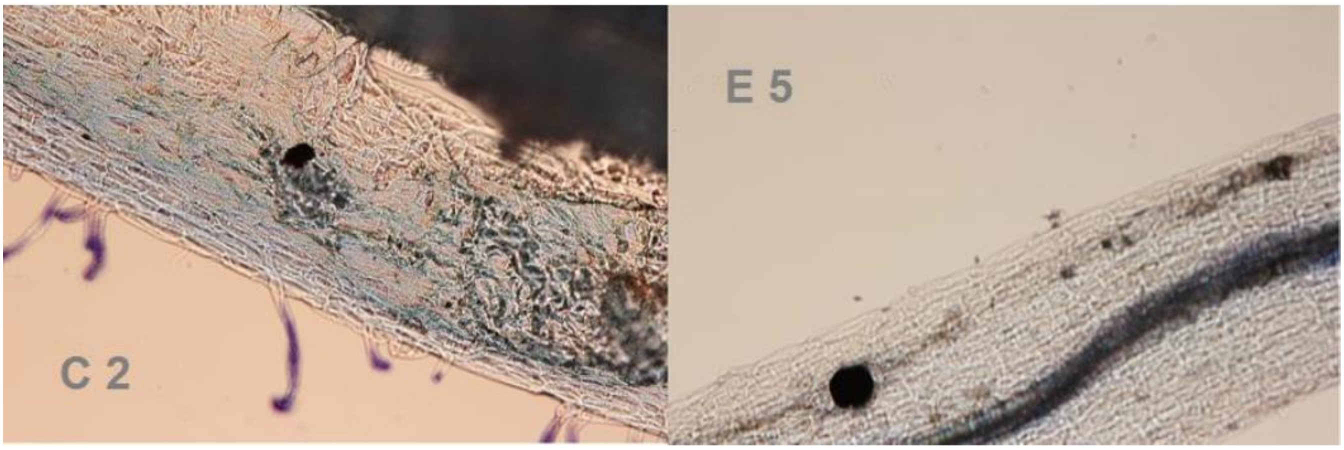
The absolute data show that while D treatment had significantly the highest bulk-soil extractable PO_4:P level, A treatment had significantly the highest level of extractable PO_4:P in the rhizosphere. The fact that there is no significant difference between A on one hand and D and E on the other, does not change this conclusion but rather confirms it. D and E come somewhere in the middle where they are not statistically different untill the two extremes they fall between have been compared significantly to each other (A with B and C). In other words, D and E must have a lower value than that of A in terms of P availability in the rhizosphere, or they would have displayed a significant difference compared to B and C as well.

6.3.3. Microscopy

Portions of the lower sections of roots, each 1 g^{-1} in weight, were sampled from two sets of each treatment randomly. Roots were kept in autoclaveable containers and boiled in KOH 10% at 120°C, left to cool down for half an hour then washed in 5% HCL for one minute then stained by Trypan Blue stain for half an hour. Under the microscope, as qualitative but not quantitative observations, hyphae could be seen and it was obvious that all roots were colonised. The roots of A and D treatments displayed fungal hotspots which could not be seen in B, C and E (see photos of the samples).


B4
B1
B4


D 2
D4
D4
D2



6.3.4. Other observations

During destructive harvest and microscopic observation of the arbuscular mycorrhizal fungi, worm hatchings were observed in all treatments except D. They appeared sparingly, with no

Pot	Earthworms			Flowers
	Hachlets	Juvenile	Adults	
A1	0	0	0	✓
A2	0	2	0	✓
A3	1	0	0	✓
A4	0	0	0	✓
A5	0	0	0	✓
B1	0	0	0	✓
B2	4	0	0	✓
B3	0	0	0	✓
B4	0	0	0	✓
B5	0	1	0	✓
C1	0	1	0	✓
C2	1	1	0	✓
C3	0	0	0	✓
C4	0	0	0	✓
C5	0	0	0	✓
D1 NPK	0	0	0	×
D2 NPK	0	0	0	✓
D3 NPK	0	0	0	✓
D4 NPK	0	0	0	✓
D5 NPK	0	0	0	✓
E1 (No P)	0	0	0	✓
E2	0	0	0	×
E3	0	0	0	✓
E4	0	1	0	✓
E5	0	0	0	✓

Table 6.11. Other observations during the experiment.

obvious impact on plant growth, and were distributed almost evenly in each treatment. They were not investigated further. The one treatment with zero hatchings also had no nodulation in the five replicates. Table 6.11 lists those observations.

6.4. Discussion

The results of the experiment were straightforward and support the hypothesis that P delivered in capsule form can significantly enhance its efficiency in the rhizosphere. They also matched the expectations that treatment D would have the highest biomass, as would be justified by the Law of the Minimum. The reason why the P efficiency capsule was the most efficient is not straight forward though. The probability that AM fungi have special affinity to Phosphorus cannot be justified by this experiment, because if so was true, AM fungi would have detected the P reservoir in the C design and would have made the best of it, which did not happen. It is also obvious that the design B of the P capsule couldn't help the plant in regards of P uptake, perhaps because P was immobilised by soil organisms while they degraded the agar-agar. The significant increase in of PO4 in the rhizosphere is stricking because A has practically received less P than all the other treatme A replication of this experiment with a suitable K and N supply to the A treatment, would definitely yield interesting results.

6.5. Conclusion

It is possible to enhance P efficiency and manipulate the rhizosphere for better P uptake by manipulating the spatio-temporal aspect of fertiliser application. Arbuscular mycorrhizal fungi seem to have no affinity for P in this experiment and enhancement is most probably due to each and all of the other potential reasons set out by Grant et al. (2004).

- The P solubilising capacity of AMF owing to phosphatase secretion.
- The higher accessibility to P, since P – owing to its high immobility in soil, must come in direct contact with the root to be uptaked.
- The capacity of AMF to change the biochemistry of the rhizosphere to allow more P bioavailability in this 1 mm critical area.

The fresh supply of P defused by the P capsule in a position accessible to arbuscular mycorrhizal fungi must then had the greatest effect in increasing the availability of extractable P in the rhizosphere. P availability in a suitable amount, might have an impact on the population of arbuscular mycorrhizal fungi as is evident by the visual observation and the data, although levels of arbuscular mycorrhizal fungi were not quantified. The application of NPK increased the biomass of the plant 91% dry weight and 137% fresh weight but suppressed nodulation and probably earthworm hatching.

The P efficiency in A treatment did not reflect significantly in its growth rate, due to the deficiency in other essential element such as N and K. If we may assume that the plant can get all its N needs through nodules, then K in this case would be the limiting nutrient.

There is no way to confirm or rule out the possibility that vermicompost encouraged arbuscular mycorrhizal fungal colonisation in this experiment, as it was not meant to be investigated at this time, and was solely used based on previous findings.

6.6. Recommendation for future investigations

- A replicate of this experiment with 6 g^{-1} K and 2-3 g^{-1} of nitrogen would yield interesting results.
- A replicate of this experience with a controlled number of earthworm eggs would yield interesting results.
- Further investigation into the impact of nitrogen or NPK on soil biota such as earthworms is needed.
- Further dynamic soil modelling integrating organic and inorganic fertilisers while observing soil biota is needed.
- Developing further designes and technologies for enhancing fertliser efficiency may have economic potential.

CHAPTER 7

Crop production and community growers

In line with discussion from Chapters 1 and 4, and to link all conclusions and findings presented in this dissertation, some space needed to be allocated to local food growers. Local food growers, specifically community growers whose 'projects' are not subsidised but are run mainly through individual effort, provide realistic views on the feasibility of food production, uninfluenced by funding guidlines or organic-food price premiums (see Pimentel, 2005).

Without specific guidlines or legal limitations, community food-growers might have (at least theoritically) the freedom to alternate between the use of organic and inorganic fertilisers, choose their seeds and decide on whether to use biological inoculants for crop enhancement. They are also likely to have a wide array of relevant-information sources. A survey was therefore prepared to explore the different perspectives community food growers hold regarding several issues - with no prior expectations of what may be the outcome. 60 hard copies of the survey were printed out and an online version was established. The online survey was forwarded to the National Allotment Society, requesting participation of members, and to the University of Edinburgh internal e-mailing lists. The online version of the survey was posted on a relevant Facebook group but was removed by its administrator who explained apologetically that people are bothered by surveys. In total, 20 responses were received, all from allotment holders in Edinburgh area.

Allotment holders were surveyed on issues like the feasibility of growing one's own food, preferences in regards of gardening/faming practices in relation to fertiliser application, their point of view relating to organic versus conventional farming practices (and food purchase) and their stand regarding GMOs technologies as a potential crop intensification solution.

7.1 Results

Soil Nutrients and Sustainable Food Survey

To explore some opinions on the opportunities and challenges facing small-scale farmers/gardeners for sustainable local food production

Number of respondents: 20

[17] face-to-face respondents [3] online respondents

Dates of participation: 18/06/2016 > 03/07/2017

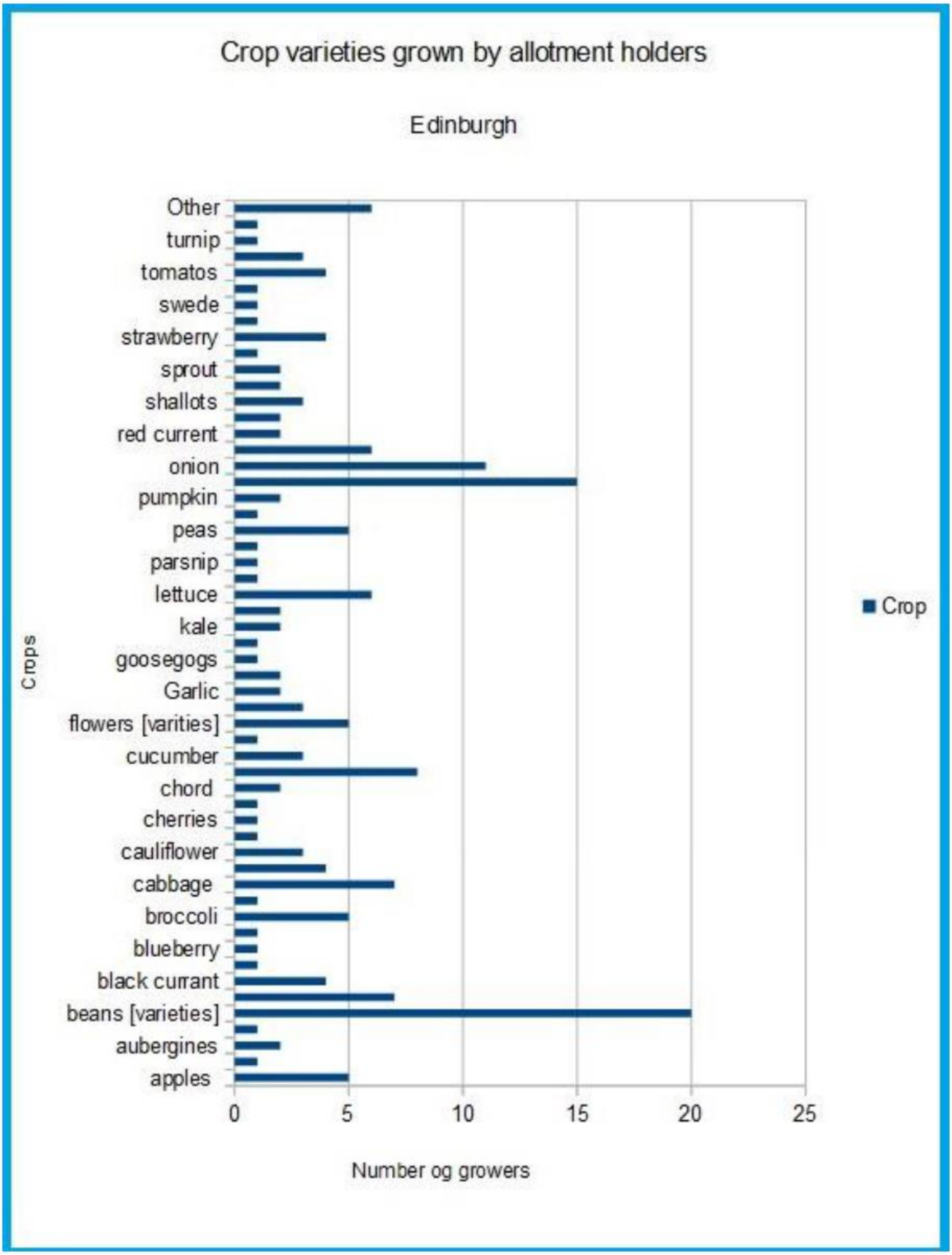
Q1: Sizes of allotments

Responses	
On site respondents	Online respondents
[3] full plots – [6] half a plots – [6] other	[2] full plots – [1] half a plot

Q2:

What are you currently growing on your allotment? Do you grow beans?

Responses


Crop varieties grown by allotment holders
Edinburgh
Other
turnip
tomatos
swede
strawberry
sprout
shallots
red current
onion
pumpkin
peas
parsnip
lettuce
kale
goosegogs
Garlic
flowers [varities]
cucumber
chord
cherries
cauliflower
cabbage
broccoli
blueberry
black currant
beans [varieties]
aubergines
apples
Crops
0
5
10
15
20
25
Number og growers
Crop

Q3: Are there any rules or regulations that must be followed by allotments holders?

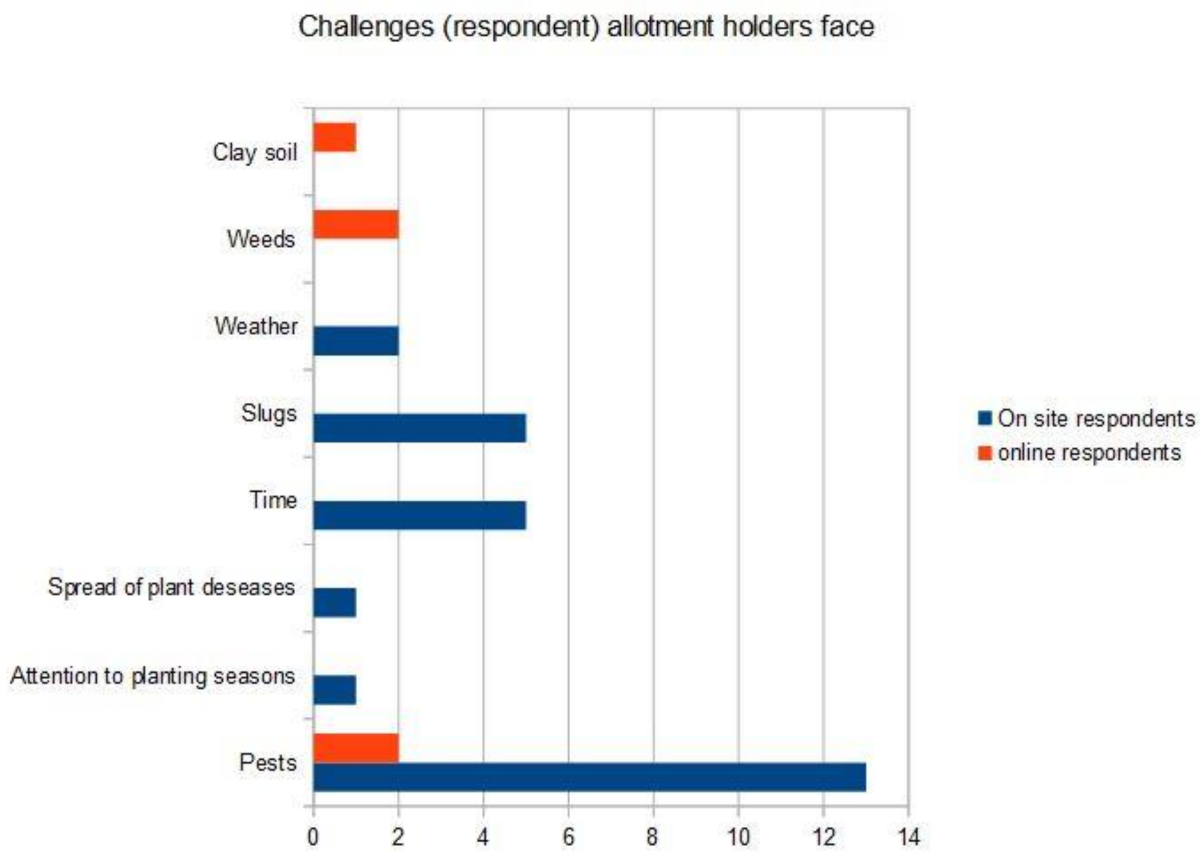
Responses			
Onsite respondents		Online respondents	
Yes	**No**	**Yes**	**No**
17	0	2	1

Q3: What is the biggest challenge for you taking care of this allotment?

Responses

Because online and on site responses may vary significantly according to local factors, a TTEST was conducted to check if there were any significant differences in responses, so as to decide whether to aggregate the data or keep them separate. Since p> was found to be 0.24, data were aggregated as follows:

Challenges	On site respondents	online respondents
Pests	13	2
Attention to planting seasons	1	
Spread of plant deseases	1	
Time	5	
Slugs	5	
Weather	2	
Weeds		2
Clay soil		1
Std error	1.68	
p>	0.19	



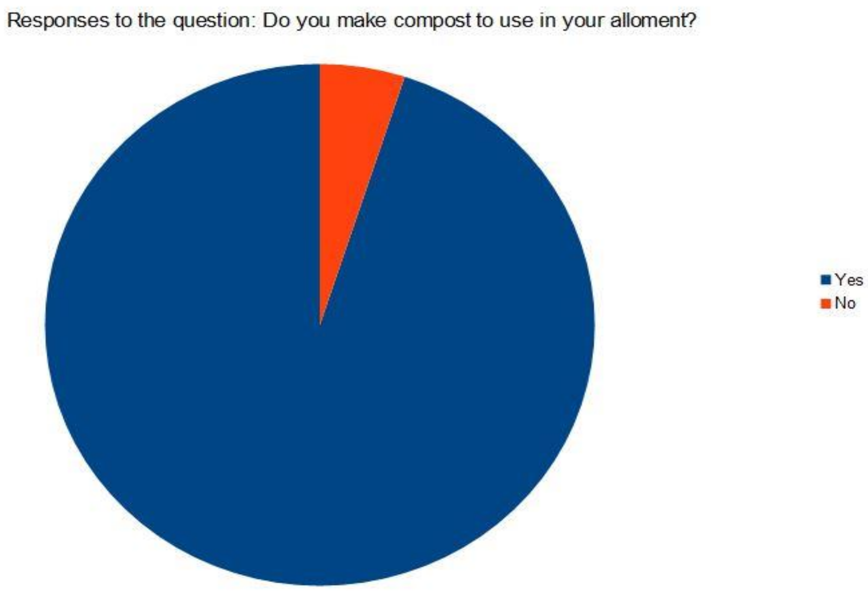
Q4 Do you use commercial compost and growbags?

On site respondents		Online respondents	
Yes	**No**	**Yes**	**No**
12	5	3	0

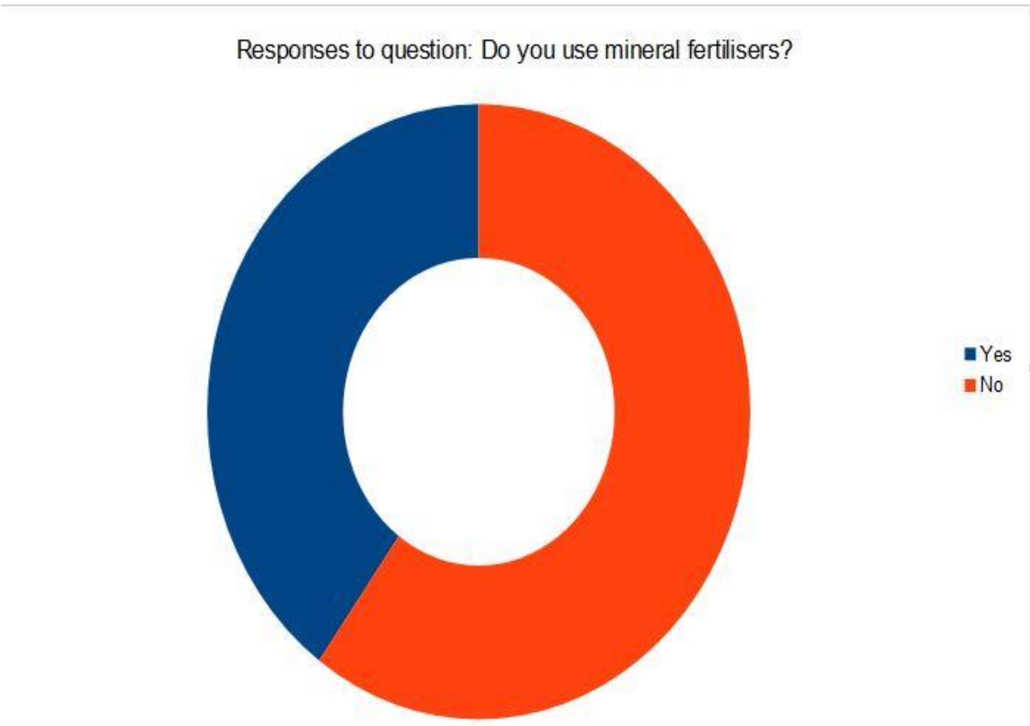
Q5 Do you make compost for use on your allotment?

On site respondents		Online respondents	
Yes	No	Yes	No
16	1 (using seaweed solution, organic chicken pellet, comfrey)	3	0

Q6 Do you use mineral fertilisers (e.g. superphosphate, potash, etc.)?



Onsite respondents		Online respondents	
Yes	**No**	**Yes**	**No**
5	12	3	0



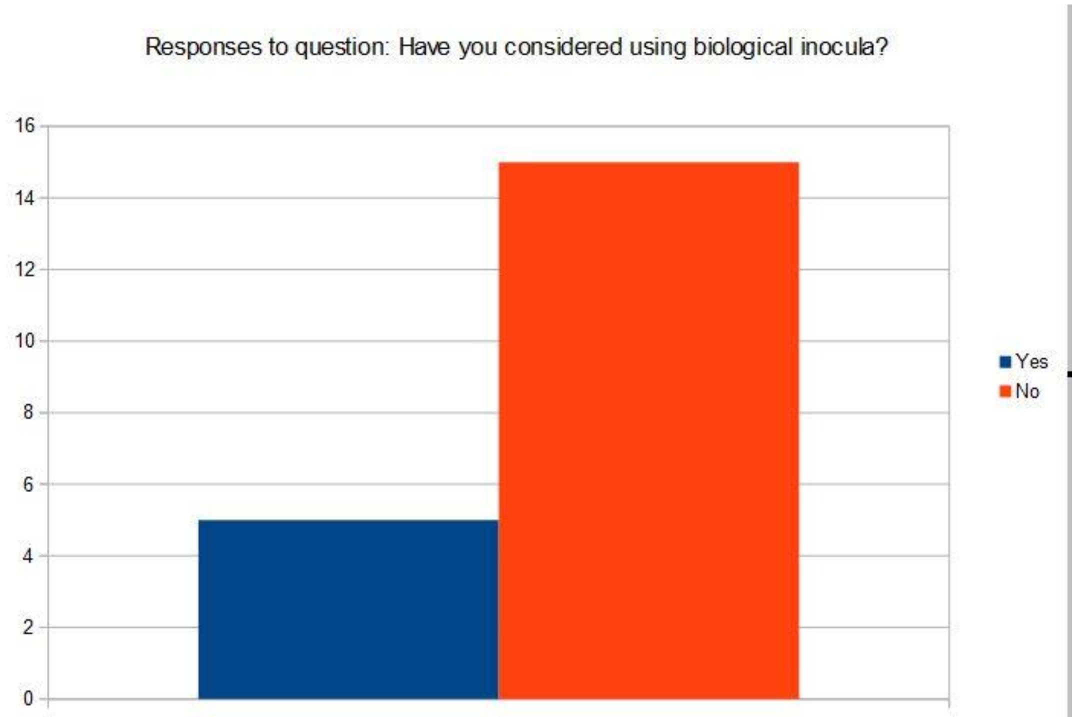
Q7 Do you or have you ever tested your soil pH and/or nutrient status?

On site respondents		Online respondents	
Yes	**No**	**Yes**	**No**
3	11	2	1

Q8 Have you ever considered buying/using biological inoculants (e.g. mycorrhizal fungi) to increase supply and availability of nutrients to plants?

Onsite respondents		Online respondents	
Yes	**No**	**Yes**	**No**
3	14	2	1

Q 8 If yes, were you satisfied with the results?



Onsite respondents		Online respondents	
Yes	**N/A**	**Yes**	**N/A**
0	18	1	1

Q 9 Roughly, how much do you spend on synthetic fertilisers and fungicides each year?

No answer =7 (35%) , 0 = 5 (25%), £1-9 = 1, £10-20 = 8 (40%)

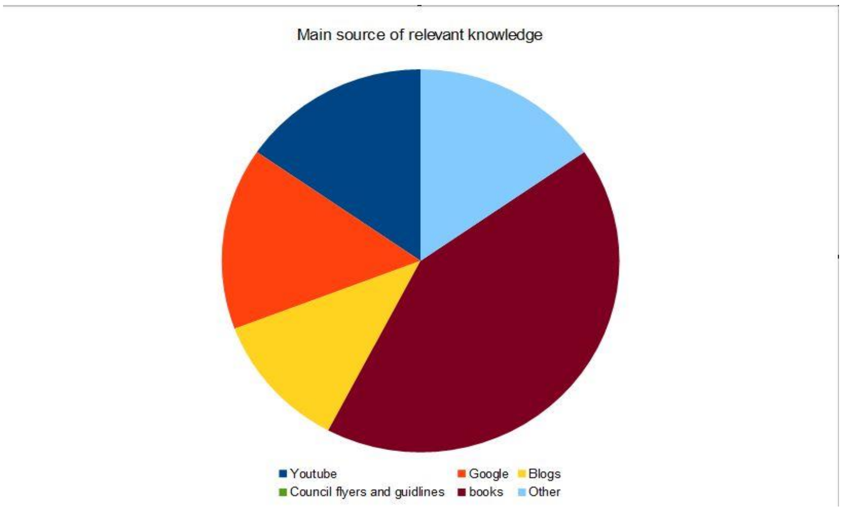
Onsite respondents				Online respondents		
No answer	**£ 0**	**£1-9**	**£10-20**	**£1-19**	**£20**	**£40**
7	5	1	4	1	1	1

Q 10 Where do you (mostly) get farming/gardening information?

Source	Onsite responses	Online responses	Total
Peers	12	2	**14**
Youtube	4		**4**
Google	6		**6**
Blogs	3		**3**
Council flyers and guidlines			**0**
Books	11		**11**
Other	2	2	**4**

Std error: 1.83
p> 0.36

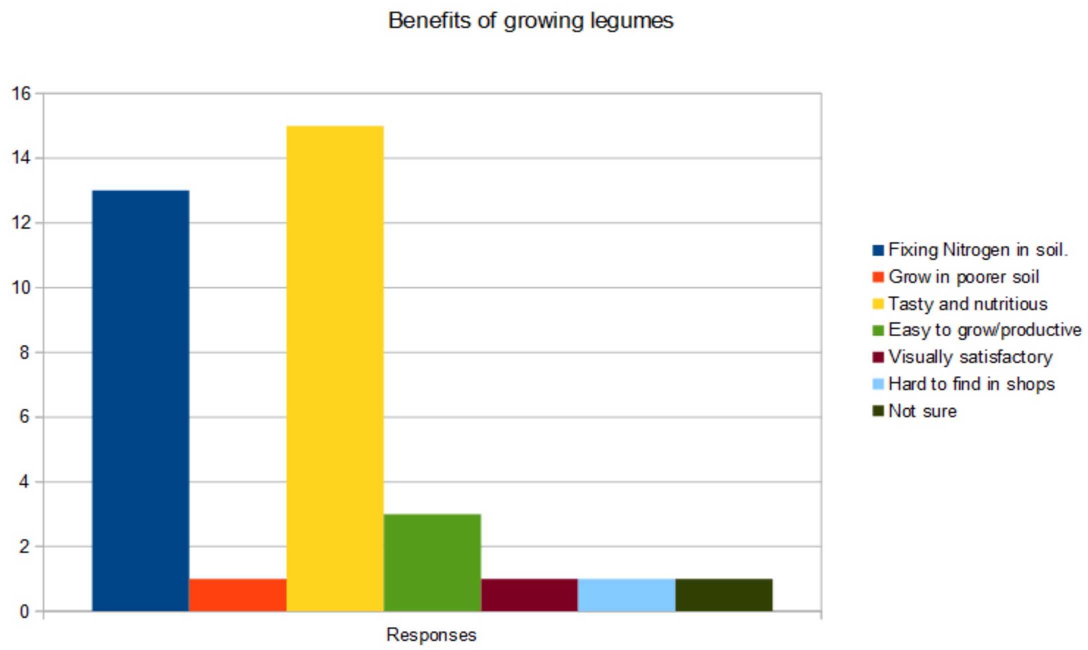
As there were no significant difference between the two sets of responses, data were aggregated.



Q 11 If yes, what are the benefits of growing legumes?

[respondents were not given choices, and thus gave their own answers – overlapping results].

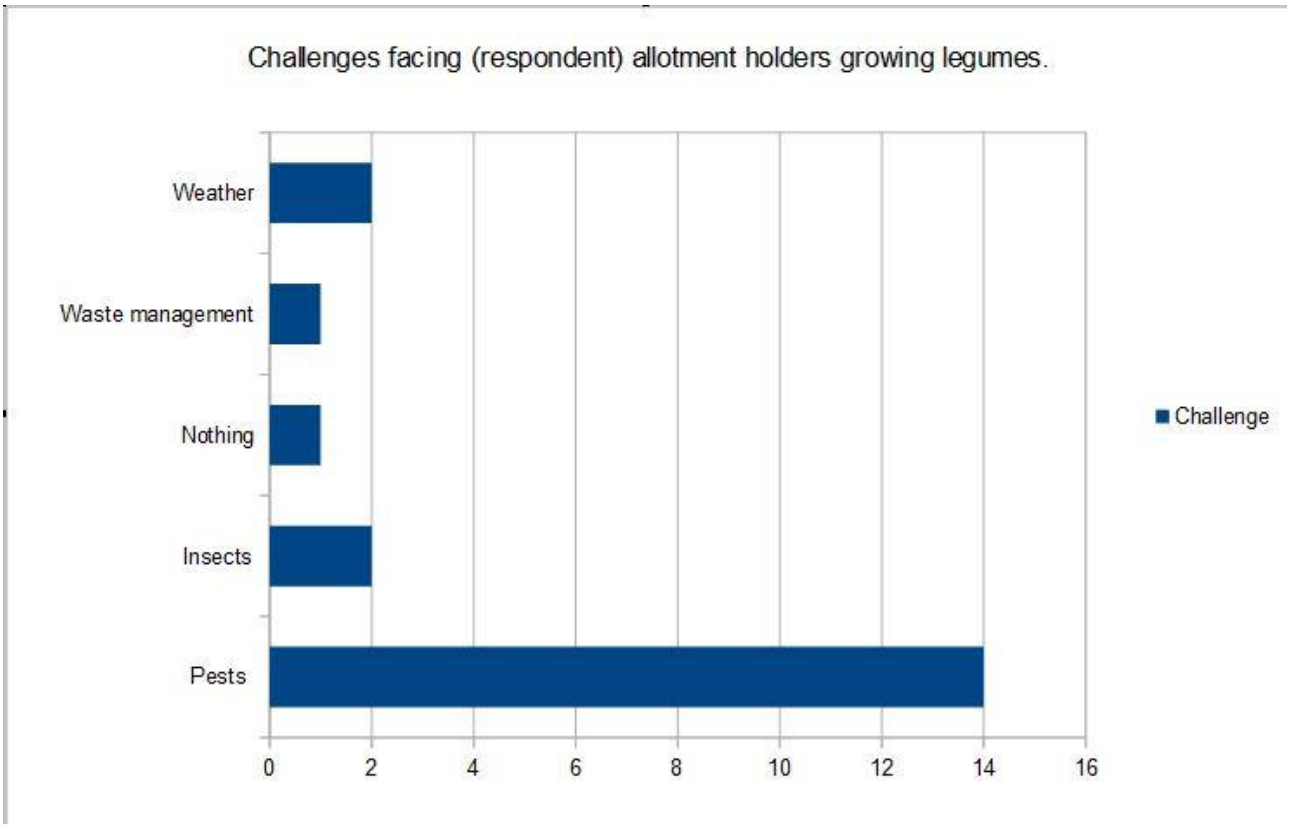
Benefits of growing legumes	Responses
Fixing Nitrogen in soil.	13
Grow in poorer soil	1
Tasty and nutritious	15
Easy to grow/productive	3
Visually satisfactory	1
Hard to find in shops	1
Not sure	1



Q12 What are the biggest challenges for growing legumes?

Challenge	Onsite	Online	Total
Weather	2		2
Pests	13	1	14
Insects		2	2
Nothing	1		1
Waste management	1		1
		p>	0.42

As there was no significant difference between the two sets of responses, data were aggregated.



Q13 Do you buy organic fruit and vegetables?

Yes	No	Sometimes
11	0	9

Q14 Does your crop yield ever exceed the needs of your household?

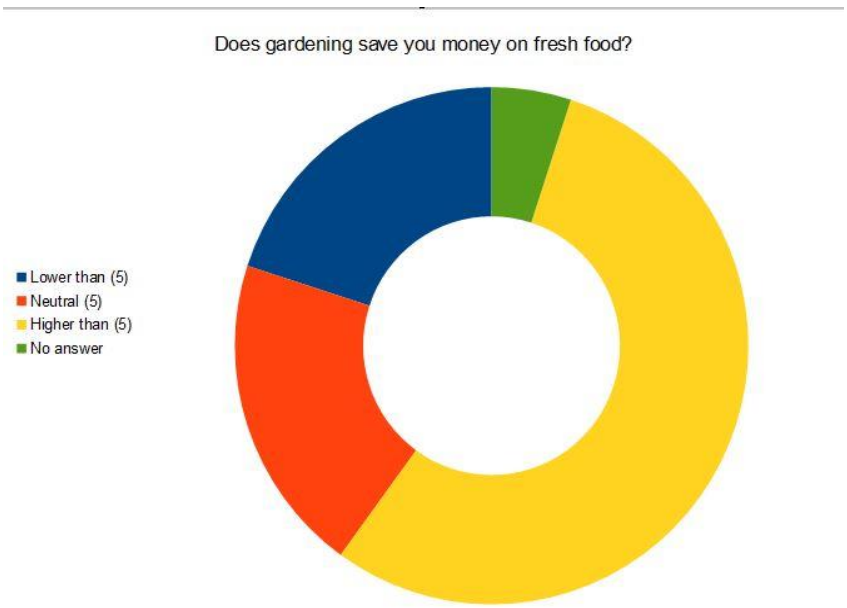
On site respondents		Online respondents	
Yes	**No**	**Yes**	**No**
13	4	3	0

Q15 On a scale from (1) to (10) -where (10) means you strongly agree and (1) means you strongly disagree,

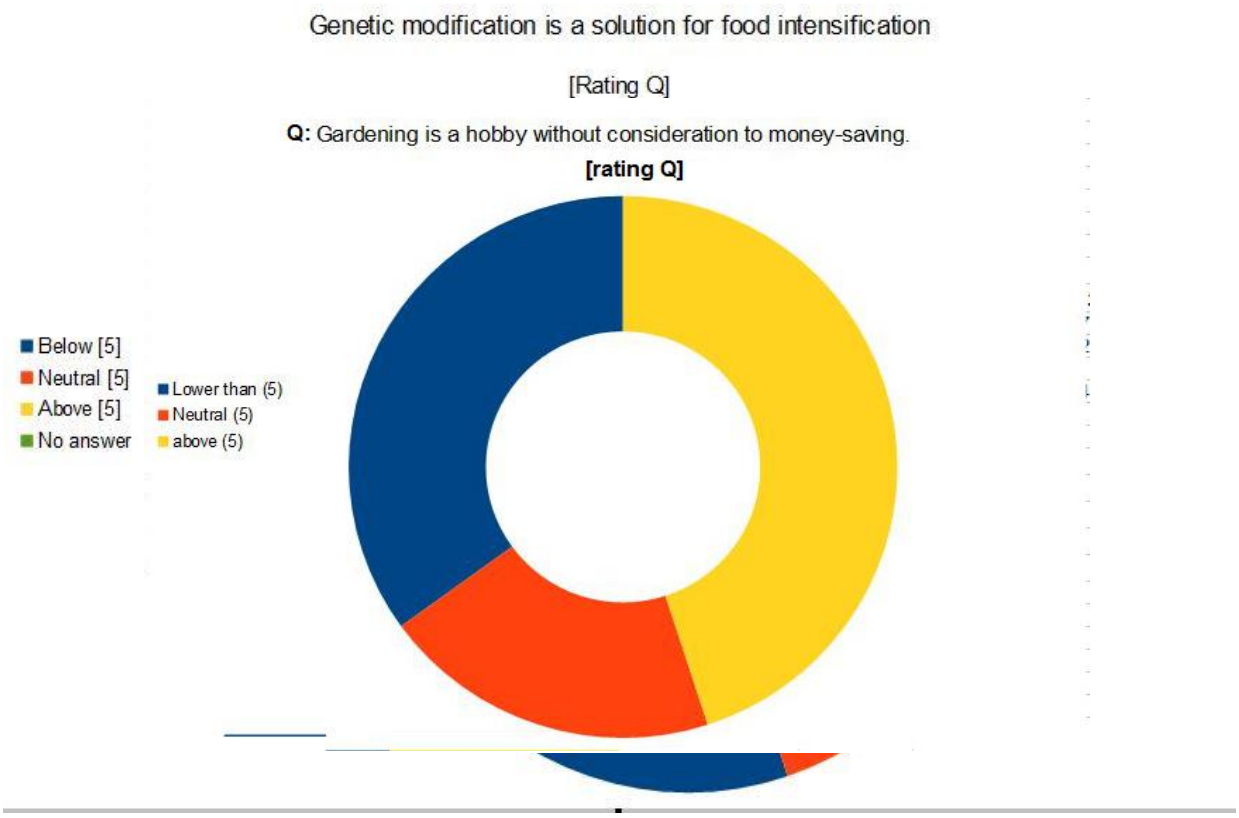
“How strongly do you agree with the following statements?”

15.1 Gardening saves me money on fresh food.

– **15.2 Gardening is just a hobby without consideration for money saving.**



- **15.3 Genetic modification is a solution for food intensification.**



– **15.4 Organic farming and biological provision of soil fertility can feed the world's growing population.**

7.2. Discussion

7.2.1. On legumes and arbuscular mycorrhizal fungi

Responses to questions 10 and 11 indicate a general awareness among allotment owners of the benefits of legume, in addition valuing them as highly nutritious delicacies. Responses to question 9, on the other hand, indicate less awareness and/or enthusiam in respect of using biological inoculants, such as arbuscular mycorrhizal fungi: 75% of respondents did not consider using biological inoculants, and only 25% said they considered it. Responses to the following question (9) however, showed that only 5% of those who said they considered using them actually did, so the real ratio for active use of biological inocula versus not using them is 95:5%. This could be because information about arbuscular mycorrhizal fungi presented to non-specialists in media such as YouTube and by commercial companies can be inaccurate.

7.2.2. On Organic versus inorganic practices

Responses to questions 5, 8, and 13.4 show that allotment holders regard to organic practices and purchases highly. Organic farming is increasingly looked at as a panacea for environmental and health problems arising from use of inorganic fertilisers and genetically modified organisms. This is also supported by responses to question 13.3.

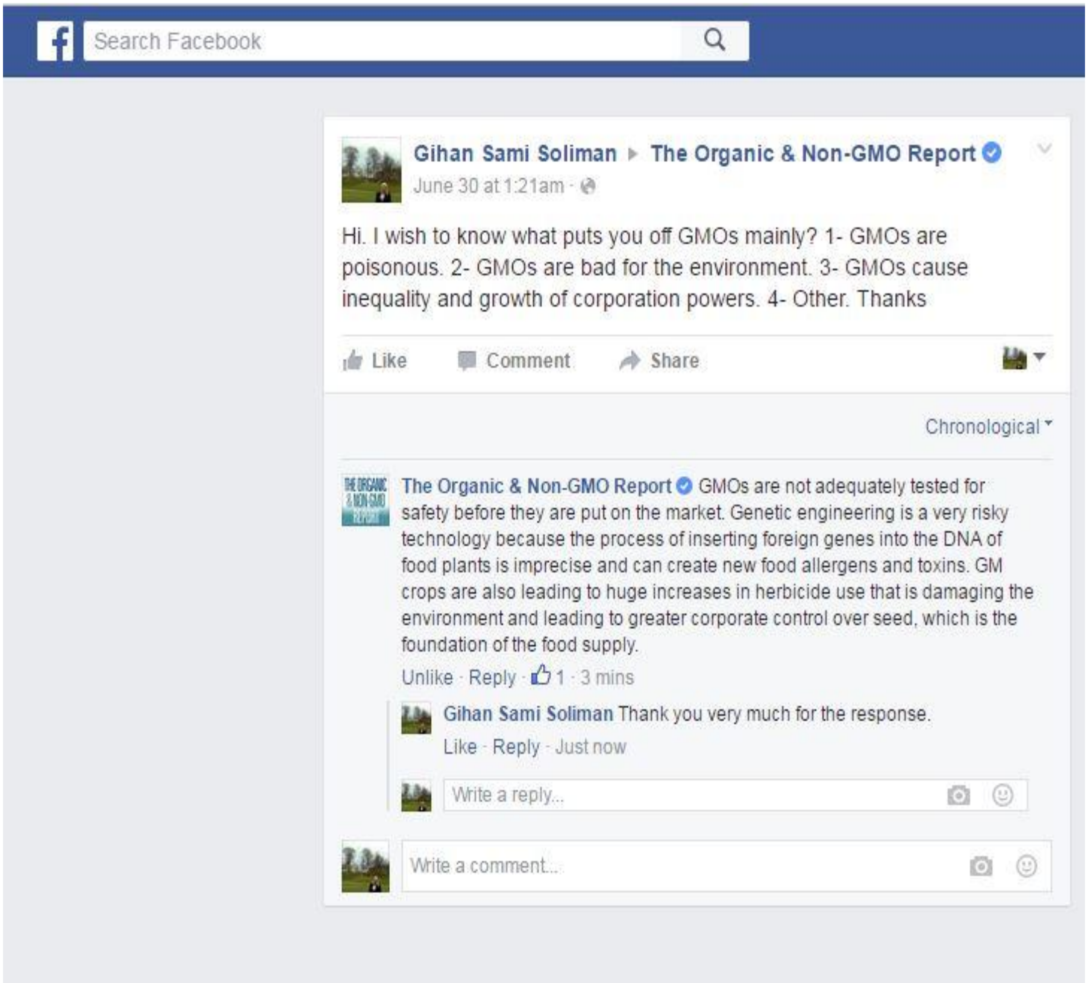
Organic farming is, however, in principle a ‘holistic system designed to optimise the productivity and fitness of diverse communities within the agro-ecosystem, including soil organisms, plants, livestock and people. The principal goal of organic production is to develop enterprises that are sustainable and harmonious with the environment' (Gold, 2004; Martin, 2009). This entails continuous research, weighing possibilites against probabilities and findings; and a permentant condition of experimenting. Nothing in organic farming should be considered intrinsically 'good' or 'bad' on its own.

A field investigation examining the differences between organic and conventional farming, in terms of energy and economic viability was conducted from 1981 through 2002 at the Rodale Institute FST in Kutztown, Pennsylvania, on 6.1 ha. (Pimentel, 2005). The investigation showed that in terms of economics, in those particular circumstances, the two systems were overall almost equal. With crop rotation, the small limitation in one crop is balanced by the abundance of another crop in organic farming, and with the organic premium price, organic farming becomes even more profitable.

In environmental terms organic farming increased water retention in soil, nitrogen and carbon reserves (Pimental et al., 2005). In terms of soil health, the organic system increases arbuscular mycorrhizal fungal colonisation, and earthworms were twice as abundant. Adding compost and other organic matter was additionally found to reduce crop diseases and environmental damage (Cook 1988, Hoitink et al., 1991). Organic farming, however, applies tons of fresh/untreated solid manures and slurries to agricultural land annually which may contain disease-causing microorganisms such as *Escherichia coli* O157 and species of *Salmonella* (Gov, UK 2012) and an increase in heavy metals concentrations. Such practices may lead to the need for bioremediation (McGrath et al., 1995 cited in Basley 2015).

In economic terms, organic systems require 35% more labour; they cost the same as conventional farming because work is distributed throughout the year and not condensed over one or two seasons as in conventional farming. However labour in organic farming is, on average, 155% higher (Sorby 2002, Granatstein 2003 cited in Pimentel, 2005), but may also range from 7-75% (Brumfield et al., 2000; Karlen et al., 1995, Nguyen & Haynes 1995 cited in Pimentel, 2010). This could only be useful to employment markets if food prices were realistic and the farming sector did not need as much subsidies and/or premium pricing. This is indirectly reflected in the survey responses of question 14 where 75% of respondents said production exceeded household needs, while most of the same respondents admitted buying organic fruits and vegetables. In question 15.1 most respondents either said farming doesn't save them money, or were neutral about it.

In respect of GMO techniques, a question was posted on a Facebook group with 928,018 followers at the time of posting. The response came next day as follows:



The tendency to view GMOs negatively is reflected in responses to question 15.3 and seems heavily influenced by concerns over corporate powers controlling the market of GMO seeds. GMO techniques, however, are not uniform and cannot all be judged by the same criteria. A technology modifying root systems, for example, is different from one enhancing plant resistence to diseases or insecticides. More research into this issue is required.

CONCLUSION

As this study examined some aspects of sustainable food intensification in its technical and socio-economic dimensions, a historical outline of the use of fertilisers was drawn. It implied use of inorganic fertilisers was a turning point in socio-economic evolution, being one of the ways the human population flourished and expanded in a few decades. Excessive use of inorganic fertilisers, however, has its down side, with serious environmental and health repercussions. This research confirms findings from previous studies which record high crop yield in a plant-growing system that integrates both organic and inorganic practices. A significant enhancement resulting from a holisitic integrated approach; that employs commercial AMF, 2% wormcast and inorganic fertilisers in different but strategic positions, combinations and release levels, was observed in the 'P-effieciency experiment' conducted in this study (although a comparative study examining the potential role of each of the organic and inorganic constituents separately, was not conducted here). The experiment outcome shows the rhizosphere can be manipulated for enhanced exchangeable-P availability and uptake, using spatio-temporal aspects of inorganic fertiliser application. Through its literature review, the study explores soil dynamics which might potentially have been involved in determining the outcome of the result, such as the relationship between/among arbuscular mycorrhizal fungi, earthworms, the soil P-index and the soil C-N budget; stressing the need for more interdisciplinary research and dynamic soil modelling. The work also explores socio-economic dimensions of food intensification and community gardening/farming using-peer-reviewed articles, a survey, social media, and popular Google search results. Implications are that food intensification has deeper socio-economical dimensions than might appear on the surface, and that neither organic nor inorganic agricultural practices can be regarded as absolutely right or wrong. Researchers should be encouraged to conduct more holistic interdisciplinary research despite challenges, and the outcome of such research should be publicised by streaming through popular communication channels such as YouTube and social media.

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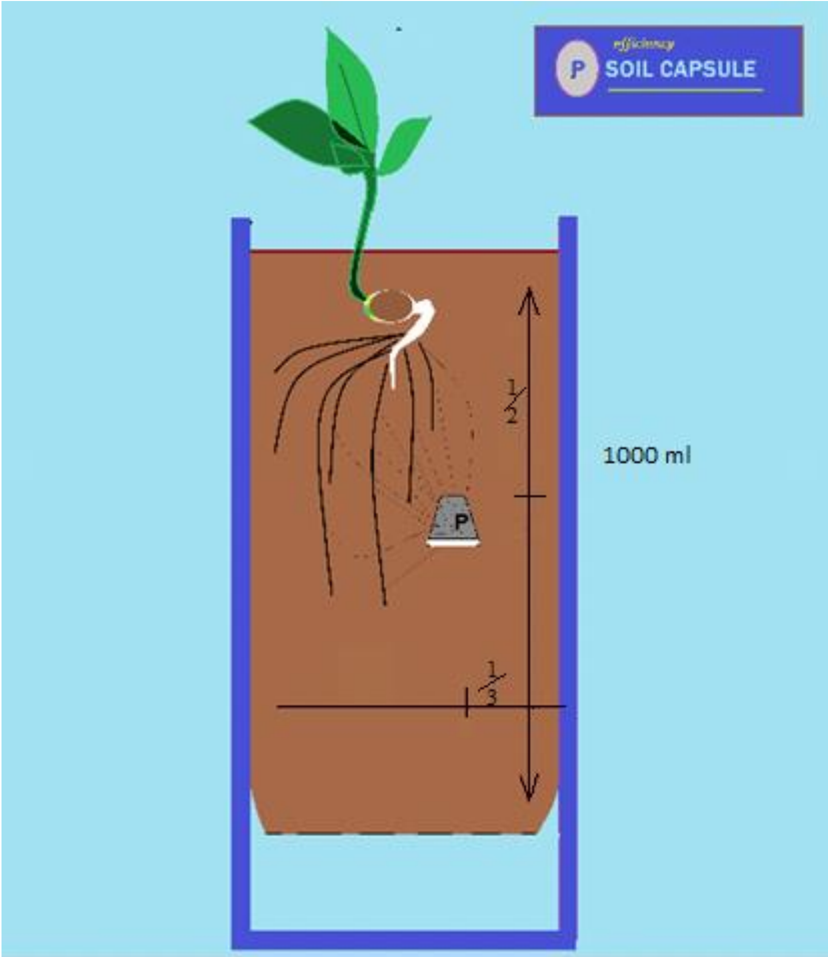
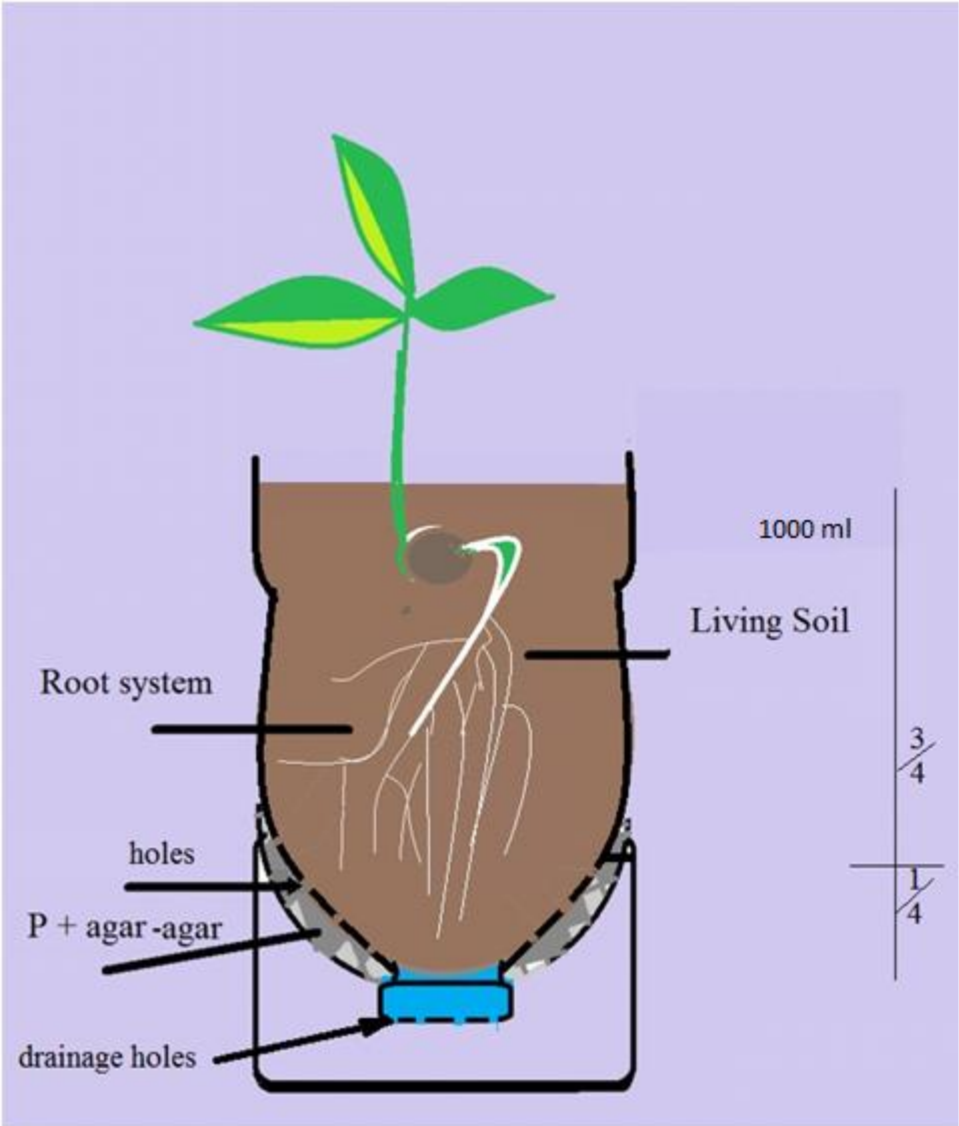
Annex:

a.



b.


1000 ml
Living Soil
Root system
3/4
holes
1/4
P + agar -agar
drainage holes