Carbon–Nitrogen–Phosphorus Stoichiometric Interactions and Microbial Community Shifts in Arachis hypogaea Rhizosphere Under Alternate Wetting and Drying Conditions

Ankit T Panchal [1*], **Ketankumar J Hinsu** [2]
[1-2] Department of Animal Biotechnology, Anand Agricultural University, Anand, Gujarat, India

* Corresponding Author: **Ankit T Panchal**

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Abstract

Background: Groundnut (Arachis hypogaea L.) rhizosphere microbial activity and nutrient cycling are regulated by soil carbon (C), nitrogen (N) and phosphorus (P) stoichiometry. Irrigation by alternate wetting and drying (AWD) causes recurrent fluctuations in soil moisture that may alter these processes.

Objective: To evaluate the effects of AWD induced soil moisture changes on the rhizosphere C:N:P stoichiometry, microbial community dynamics and groundnut productivity.

Methods: Published studies on AWD irrigation, soil nutrient stoichiometry, microbial ecology and groundnut cultivation were synthesized and evaluated.

Results: AWD alters the physicochemical properties of the soil, which in turn influences the availability of C, N and P and modifies the microbial community composition. Rewetting events stimulate microbial activity via the Birch effect, alter enzyme production, affect Bradyrhizobium-mediated nitrogen fixation, and enhance phosphorus release from soil aggregates. Such changes affect nutrient-use efficiency, root growth and pod yield.

Conclusion: Changes in groundnut rhizosphere C:N:P stoichiometry driven by AWD significantly influence microbial functions and nutrient cycling in groundnut systems. Combining advanced microbial analyses and precision water management can enhance productivity and sustainability under variable climatic conditions.

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1. Carbon–Nitrogen–Phosphorus Stoichiometry in Agricultural Soils

1.1. Fundamental Principles of Ecological Stoichiometry

Ecological stoichiometry is the study of chemical element balance in ecological interactions, and the implications of that balance on organism physiology, population dynamics, and ecosystem function. A fundamental principle of ecological stoichiometry is that all living things are made up of elements; therefore, the ratio of Carbon (C): Nitrogen (N): Phosphorus (P) in an organism (and its environment) governs its rates of metabolism, efficiency of growth, and ability to acquire nutrients. The other major concept in stoichiometric theory is homeostasis (the idea that organisms maintain stable internal ratios of elements regardless of variability in the availability of those elements in their external environment). Most animals exhibit strict homeostatic regulation while many plants and some microorganisms exhibit flexible stoichiometry – this distinction between how organisms regulate their internal elemental composition is an important foundation of stoichiometric theory (Sterner *et al.*, 2002) [3]. Key to stoichiometric research conducted on terrestrial soils are the ecological factors that regulate stoichiometric homeostasis – variations in substrate quality, fluctuating environmental conditions, and interactions between biotic and abiotic factors present constant challenges to the principles of stoichiometric homeostasis in soils.

The initial development of the Redfield ratio was based on marine phytoplankton, but this ratio (C:N:P) of 106:16:1 has been adapted to terrestrial-based microbial communities and is now known from global meta-analyses of C:N:P ratios of microbial biomass averaging about 60:7:1 (Cleveland and Liptzin, 2007) [2] and soil organic matter C:N:P ratios having an average of about 186:13:1 (Xu *et al*., 2013) [4]. Both of these ratios are not fixed values but rather are representative averages of a mean with varying degrees of variability based on climate, parent material, land use and vegetation type (Tian *et al*., 2010) [30] thus indicating that the ecological implications for rhizosphere ecology are that deviations from these mean ratios, or "stoichiometric imbalances", impose metabolic limitations or constraints to microbial communities, resulting in the formation of physiological adaptations dependent upon changes in uptake stoichiometry, increases in overflow metabolism, and variation in levels of enzyme production (Zeng *et al*., 2017) [5].

1.2. Importance of Carbon, Nitrogen, and Phosphorus Interactions in Soil Systems

In ecosystems and agricultural systems, the three macronutrients (C, N, and P) are involved in biogeochemical cycles that are linked together. Biologically, carbon is both the energy source and a component of biomass (microorganisms), nitrogen is the preferred fuel for protein synthesis, and phosphorus plays a role in energy transformations as the primary component of ATP, nucleic acids, and phospholipid membranes (Vitousek *et al*., 2010) [31]. The interaction of these nutrient cycles means that deficiencies or excesses of one nutrient affect the availability of another as a result of microbial stoichiometric adjustments (Elser *et al*., 2007) [1].

Synthetic fertilisation usage in agricultural soils has increased anthropogenic additions of nitrogen and phosphorus (N and P), which disrupted natural stoichiometric relations between carbon (C), N and phosphorus (P) that existed previously in undisturbed terrestrial ecosystems. For instance, if there is increased N availability without an increase in C inputs, microbial community shifts toward copiotrophic strategy, exhibit reduced decomposition of recalcitrant organic matter from soil and in the end the depletion of soil organic C pools. In areas of Arachis hypogaea with highly weathered soils and little P, microbial population growth is limited by P, even when both N and C are in excess, leading to greater allocation of energy into producing phosphatase enzymes and developing mycorrhizal associations than would typically be observed if sufficient P were present (Vitousek *et al*., 2010) [31]. The knowledge of these interdependent dynamics is critical when developing nutrient management plans for healthy and productive soil ecosystems.

1.3. Mechanisms Controlling C:N:P Balance and Nutrient Coupling

There are many mechanisms that regulate the C:N:P balance in soils when they go off balance and all of these are interdependent and occur at both short and long time frames as well as different spatial scales. Microorganisms can maintain a reciprocal C:N:P ratio, or stoichiometric homeostasis, by taking up nutrients at different rates, consuming luxuriously, storing nutrients in cells and excreting surplus amounts through overflow metabolism (Sterner *et al*., 2002) [3].

If a microbe's growth is dependent on a substrate C:N:P ratio, when there is a significant discrepancy between the two, there may be energetic and biosynthetic imbalances and this will be reflected by changes in the rate of growth, the ratio of respiration to growth and ultimately, the composition of the community will shift (Zeng *et al*., 2017) [5]. There can be complementary resource use among community members that have different stoichiometries, which means the imbalance at the community level may help to stabilize the system at the functional level via functional redundancy and cross-trophic feeding (Zhou *et al*., 2010) [24].

The primary source of carbon and nitrogen in the rhizosphere for microbial populations comes from plant-derived (allochthonous) inputs such as root exudates, mucilage, senescent cellular material, and litter debris. The quantity and stoichiometry of these inputs varies greatly depending on plant species, the stage of growth, nutrient status at the time of growth, and abiotic environmental stress (Shen *et al*., 2013) [14]. In nitrogen fixing legumes like the groundnut, the additional nitrogen contribution from biological nitrogen fixation can fundamentally alter soil nitrogen and phosphorus balance, potentially transitioning the soil system from N-limited to P-limited after successive cropping. Thus, the relationship between the nitrogen level in the plant, the activity of the nitrogen fixing bacteria, and the nitrogen and phosphorous ratios in the soil constitutes a dynamic feedback loop that has large implications for long-term management of soil fertility.

1.4. Soil Nutrient Limitation Theories and Stoichiometric Homeostasis

Nutrient limitation in soils refers to both traditional approaches where researchers have attempted to determine what was the single

most limiting nutrient for microbe or plant growth at a certain time, along with more recently developed approaches using stoichiometric frameworks that have recognised that the limitations an organism faces will depend on many different factors that may occur at the same time (e.g. quality of substrates, levels of moisture available in the environment, characteristics of the microbial communities) and that in many cases, can change over time. Clay and others have recently developed an approach called ecoenzymatic stoichiometry that systematically evaluates the relative activity of C, N and P acquiring enzymes to evaluate the presence or absence of limitations of nutrients in soils. When the C:N enzyme ratio is greater than approximately 1:1 (BG:NAG), it indicates that nitrogen is limiting; likewise, if the C:P enzyme ratio (BG:phosphate) deviates from the norm, it indicates that phosphorus is limiting.

Microbial communities show a relative rather than an absolute form of stoichiometric homeostasis (Xu *et al*., 2013) [4] through plasticity in their physiology when faced with moderate stoichiometric imbalances. However, in cases of severe imbalance, such as during pulsed organic carbon additions associated with AWD rewetting events, microbial communities exceed the limits of their ability to maintain homeostasis, resulting in both transient and ecologically significant disturbances to community structure, metabolic functions, and efficiencies of nutrient cycling. The degree of homeostasis that microbial communities exhibit also varies with the phylogenetic composition of the community members themselves; (broadly) oligotrophic taxa correlate with stringent stoichiometric homeostasis, whereas copiotrophic taxa show a greater degree of flexibility in stoichiometric ratios (Bastida *et al*., 2016) [27].

1.5. Biogeochemical Significance of Nutrient Ratios

Specific C:N:P ratios are significant for ecosystem health, as well as how they affect biogeochemical cycles and processes within an ecosystem. Key stoichiometric ratios and their ecological significance in the agricultural soil system (found in Table 1). The C:N ratio of soil organic matter has been used extensively as a predictor of both decomposition rates of organic matter (decomposition dynamics) and net N mineralisation. Ratios of approximately <20 (C:N) typically mean net N turns to inorganic form via mineralisation, while ratios of ≥25 generally mean net N is immobilised in microbial biomass, causing a reduction in plant-available N temporarily (Brady and Weil, 2016) [11]. In addition, the N:P ratio indicates relative nutrient limitations for primary producers as well as decomposers; N/P ratio <10 indicates nitrogen limitation, while N:P ratio >30 indicates phosphorus limitation. Therefore, these ratios can affect ecological processes such as denitrification efficiency, and mycorrhizal colonisation intensity, by crossing critical thresholds (Vitousek *et al*., 2010) [31].

Table 1: Ecological significance of soil C:N:P stoichiometric ratios and nutrient interactions in agricultural soil systems.

Stoichiometric Ratio	Ecological Parameter	Significance and Mechanism	Agricultural Relevance
C:N ratio (~10:1)	Carbon and nitrogen balance	Regulates organic matter decomposition and nitrogen immobilization/mineralization; narrow ratios favour net N release	Soil microbial biomass metabolism
C:P ratio (~186:1)	Carbon and phosphorus coupling	Controls P immobilization during organic matter decomposition; governs microbial P demand relative to substrate quality	Phosphorus cycling efficiency
N:P ratio (~13:1)	Nitrogen and phosphorus balance	Indicates relative nutrient limitation; low N:P suggests N limitation; high N:P suggests P limitation for microbial growth	Microbial growth stoichiometry
Microbial biomass C:N (~6:1)	Microbial stoichiometric homeostasis	Microbial communities maintain relatively stable intracellular ratios despite varying substrate stoichiometry via flexible uptake and overflow metabolism	Community-level nutrient regulation
Enzyme C:N:P ratio	Ecoenzymatic stoichiometry	Ratios of beta-glucosidase, urease, and phosphatase activities reflect relative microbial C, N, and P acquisition demands and substrate limitation	Nutrient acquisition strategy
Litter C:N ratio	Organic matter quality index	High litter C:N (>25) promotes microbial N immobilization and retards decomposition; low ratios (<15) stimulate net mineralization	Decomposition dynamics

1.6. Short-term versus Long-term Stoichiometric Regulation Processes

There is a significant difference between short-term changes and long-term changes in soil stoichiometry research; short-term changes occur quickly and can be reversible, while long-term changes can take years to happen and typically cannot be reversed.

Short-term stoichiometric changes are largely driven by the physiological responses of microorganisms, and these physiological adjustments happen over the course of hours to days in response to pulses of substrates or environmental changes. The physiological changes that occur as a result of the addition of new substrate to the soil (or rewetting dry soil) create the Birch Effect, which is the large release of CO2, N, and P that occurs at the same time. These physiological adjustments are the primary way through which the effects of the alternate wetting and drying cycle relate to the seasonal availability of nutrients.

Long-term stoichiometric changes occur over the course of seasons or decades and involve gradual changes in the quality of the organic matter, the composition of the microbial community, and the nature of the interactions between the plants and soil microorganisms. Repeated alternate wetting and drying cycles will increase the amount of carbon in the soil in unmetabolizable forms due to the mineralization of the more metabolizable fractions of organic matter during the cycles, which will increase the C:N ratio of the organic matter, and will lead to a shift in the microbial community from a metabolically efficient community to a metabolically inefficient community that is dominated by oligotrophic Actinobacteria.

2. Rhizosphere Ecology and Functional Characteristics of Groundnut Systems

2.1. Morphological and Physiological Characteristics of Arachis hypogaea

Arachis hypogaea L. (commonly known as peanut) belongs to the Fabaceae family of legumes, and is an annual plant with no set growing season because it produces seeds over many months (self-seeding), native to South America. There are two primary types (virginia - bunch and spreading; spanish / valencia) of Arachis hypogaea, which vary in growing habit/morphology, maturity length, and performance agronomically, depending upon location (Boote *et al*., 2003) [13]. An interesting features of Arachis hypogaea is that although the flowers that produce pods/outside of Arachis hypogaea produce below ground after the flower has been fertilized and on the stem, then elongates due to gravity (geotropic) away from the base until formation of the inside of the peanut occurs. Therefore, both physical characteristics that occur at the peanut pod-soil interface and chemical characteristics of the soil are of extreme importance in determining yield during the season.

Arachis hypogaea is also a C3 type of photosynthesis, therefore Arachis hypogaea has had the opportunity to develop moderate drought tolerance due in large part to osmotic adjustment and the ability to regulate gas exchange as well as partitioning of photosynthetic reserves (assimilates) to root growth during times of limited soil moisture (Marschner, 2012) [6]. The nitrogen economy for Arachis hypogaea relies heavily on biological nitrogen fixation (BNF) through an association with Bradyrhizobium spp., predominantly Bradyrhizobium japonicum and B. elkanii, with lesser amounts of Rhizobium and

Mesorhizobium spp. (Salvagiotti *et al*., 2008) [12]. Under the proper environmental conditions for BNF, 80- 200 kg N ha-1 season-1 can be supplied to Arachis hypogaea, thus resulting in a significantly lower nitrogen requirement for the Arachis hypogaea plant compared to other legumes, in addition to modifying the ratio of nitrogen contained in the rhizosphere of Arachis hypogaea relative to the rhizosphere of nonlegumenous species.

2.2. Root Architecture and Rhizosphere Characteristics

In the root system of A. hypogaea, there is one taproot which goes deep, between 1.5m and up to 2m under good conditions and there will be a lot of other roots that are more horizontal than vertical and all of the other roots are close to the surface (in the top 30cm). There are root hairs on all the lateral roots and these make a much bigger surface area for taking up nutrients and water. The area of soil around the plant that has been affected by the roots and their activities is called the rhizosphere and is considered to be an area of soil around the root that has been altered at a distance of about 1-3mm from the root. Compared with bulk soil, the rhizosphere is different physically, chemically and biologically. The pH of the rhizosphere is usually lower than that of bulk soil due to the release of protons as a result of taking up ammonium and producing organic acids; this reduction in the pH of rhizosphere can increase phosphorus (P) solubilisation from mineral phosphate sources in calcareous or slightly alkaline soils.

Root nodule formation adds another dimension of complexity in the ecology of groundnut rhizospheres. Nodules tend to form preferentially on lateral roots above the taproot and due to the localised build up of fixed nitrogen around senescent nodules create hotspots with elevated nitrogen:phosphorous ratios in the rhizosphere. These spatial stoichiometric gradients generate corresponding gradients in microbial community composition and activity which is illustrated by rhizosphere molecular work that has documented systematic radial changes in taxon-specific abundances from the surface of the roots outward (Mendes *et al*., 2013) [9] (Singh *et al*., 2004) [10].

2.3. Root Exudation and Rhizodeposition Dynamics

The substances produced by plant roots that are released to the soil are called root exudates. Plant roots are continually releasing a wide variety of chemical substances (e.g., primary metabolites, secondary metabolites, and macromolecules), through several mechanisms (e.g., passive diffusion, vesicle transport, membrane channels) from living root cells. The composition of the root exudates of Arachis hypogaea changes as the plant develops. For instance, during germination and seedling establishment, there are abundant sugar-rich root exudates with low nitrogen to phosphorus ratios. However, during reproductive stages, greater quantities of nitrogen containing root exudate are produced because of the high nitrogen needs of the pod as it develops. In addition to chemical root exudates, rhizodeposition includes root border cell sloughing, root cap mucilage, and root tissues that have died. The more recalcitrant materials produced by rhizodeposition provide the principal carbon source for soil organisms responsible for decomposing material in the rhizosphere. Further, the carbon to nitrogen ratios of soil located just below the root surface and adjacent to root exudates (rapidly broken down) are substantially lower than the carbon to nitrogen ratios of soil adjacent to root sloughed cells, mucilage, and senescent root tissues (recalcitrant rhizodeposition). While there is currently no acceptable method for quantifying rhizodeposition, isotopic dilution studies have shown that A. hypogaea allocates 20-35% of its net photosynthesis to underground processes and 10-20% of this is released as root exudates. In conditions characterised by moisture stress or alternating wet/dry conditions, the composition of the root exudate changes to favour the production of osmolytes, reactive oxygen scavengers, and signalling compounds (e.g., strigolactones) that can influence mycorrhizal and bacterial interactions.

2.4. Plant-Microbe Interactions in Legume Systems

Leguminous crops are home to a variety of diverse and active microbial populations (including Arachis hypogaea) that are influenced greatly by the different nitrogen cycles and chemical compositions of these plants. In addition to the more widely recognised relationship between Rhizobium and legumes, groundnut roots are also colonised by arbuscular mycorrhizal fungi (AMF), plant growth beneficial rhizobacteria (PGPR) and a number of free-living nitrogen-fixing bacteria such as Azospirillum, Azotobacter and Gluconacetobacter species. These multi-trophic interactions have resulted in the creation of complex and functionally redundant microbiomes/communities, which can be severely affected by moisture levels, soil physical/chemical properties and cropping system management strategies.

There are many factors that influence the chemical signalling processes involved in the nodulation of legumes by rhizobia (e.g., the exchange of flavonoids and Nod factors). For example, the signalling processes that govern the relationship between legumes and rhizobia can be sensitive to soil moisture and soil pH. As a result of this, the fluctuation of moisture and pH levels due to AWD may also create fluctuations in nodulation efficiency even where sufficient Bradyrhizobium populations exist in the soil. In addition, the use of strigolactone hormone signalling (which initiates the establishment of AMF) may also be affected by both low P levels and low water levels, thus AWD may enhance the establishment of AMF at intermediate amounts of available water but may inhibit the establishment of AMF at either very low (drought) or very high (flood) available water conditions.

2.5. Biological Nitrogen Fixation and Nutrient Acquisition Strategies

In the root nodules of groundnut plants, the nitrogenase enzyme complex reduces atmospheric N2 to NH4+, an important source of nitrogen for the plants, using 16 ATP and 8 electrons for each N2 molecule fixed. Environmental factors like soil oxygen concentration, water availability, and the carbon supplied from photosynthate also regulate the process of Biological Nitrogen Fixation (BNF). Problems with nodule function may occur in Alternate Wetting and Drying (AWD) management systems. During flooding events, oxygen levels in the nodule cortex are low due to insufficient oxygen availability. During drying events, the carbon supplied to the nodule via the phloem is reduced and the nodule is hydraulically isolated from the surrounding root cortex. This means that if not properly managed in AWD systems, Arachis hypogaea may experience periodic nitrogen deficiencies, which could negatively impact the stoichiometry of the species in the rhizosphere.

A suite of nutrient acquisition strategies developed by A. hypogaea modulates rhizosphere stoichiometry beyond just

BNF. Roots exude greater amounts of citrate, malate and oxalate when access to P is low due to the prevalence of Al and Fe oxide mineral forms in the highly weathered Alfisols and Ultisols typical of tropical groundnut growing regions. Phosphatase enzymes produced by both the roots of the plant as well as those from microbes hydrolyze the organic P ester bonds in organic matter into dissolved inorganic P. At the soil surface beneath the roots, AMF hyphae extend into larger volumes of soil than roots can and are able to provide additional dissolved inorganic P to the host plant in exchange for photosynthates. These are considered the mechanisms used by various plants to acquire P when it is limited and thus collectively represent the highly integrated, plant and microbe cooperatively functioning P acquisition strategy. Further, all of this occurs in conjunction with the moisture dynamics within the soil which are controlled by AWD as those dynamics substantially influence the success of this entire process.

2.6. Rhizosphere Processes Across Different Growth Stages

The structure and ecological characteristics of the microbial community in the rhizosphere of A. hypogaea can vary greatly across the stages of development of the crop: vegetative growth, flowering, pegging, pod fill, and the final maturity of the fruit/seed. There is a large amount of fresh root surface area available for colonization by fast-growing Proteobacteria during the vegetative stage due to the rapid growth of roots and lateral roots (Mendes *et al*., 2013) [9]. Flowering and pegging are the two stages of highest metabolic demand, when there is maximal exudation from the roots and maximal N2-fixing activity of nodules present, and the levels of C and N in the rhizosphere are considerably more favourable for microbial growth (low C:N ratio and high microbial biomass). When pods are filling, assimilate now is being distributed primarily to the pods and, consequently, there is a reduction in the C input to the rhizosphere, which may temporarily limit the available C to the microorganisms in the rhizosphere (Boote *et al*., 2003) [13]. The elucidation of the dynamics at each of these stages is critical for interpreting the data collected from AWD experimental studies that have collected the data at a single time point, as the timing of collection relative to the developmental state of the crop can greatly influence treatment effects on the stoichiometry and composition of the community in the rhizosphere.

3. Alternate Wetting and Drying: Principles and Soil Moisture Dynamics

3.1. Principles and Mechanisms of Alternate Wetting and Drying Systems

The water management technique known as Alternate Wetting and Drying (AWD) was originally designed for use with lowland rice, but it is now being used on trial with other types of crops grown in upland areas. Alternate Wetting and Drying typically involves alternating soil moisture states between being close to saturating to being at a moderate water deficit, at specified intervals, usually between 3 and 14 days apart depending on the soil texture, evapotranspiration demands, and the growth stage of the crop (Brady and Weil, 2016) [11]. Agricultural research has shown that rice crops grown using AWD can save 15-30% of their total water use with no significant yield loss due to decreasing percolation losses and transpiration and reductions in methane emissions from an anaerobic environment occurring as a result of the wetting and drying process.

The way that AWD is applied to dryland crops (like peanuts) differs from using AWD when growing rice; when AWD is used with dryland crops there are never full wetting cycles and there is some fluctuation between field capacity (generally -33 kPa) and deficit threshold amounts (-80 to -150 kPa depending on the drought tolerance of the crop and its growth stage). The drying rate between applications will depend on how fast water can move through the soil profile, roots take up water, and how moisture evaporates from plant leaves and vapor can be found in the atmosphere. In reality, the actual use of AWD in the field (as opposed to in controlled research plots) will be very variable in terms of exactness of application when using surface irrigation (water applied directly to plants) as opposed to when precipitation events occur (rain happens). Rainfall and rainfall intensity in dryland production puddle or wetland production puddles create large differences between what happens in the field and what is happening around the rhizomes of the crop.

3.2. Influence of Fluctuating Moisture Regimes on Soil Physical Properties

The ongoing change between wet and dry states produces physical changes in soil structure that are both quantifiable and physiologically important (Table 2). The most immediate effect of quickly wetting dry aggregates is slaking, or the action of water causing a portion of the aggregate to swell, and the explosive escape of air trapped inside leads to having to mechanically break apart aggregate (refer to photo) by the combination of swelling of clays and the mechanical disruption of the air squeezing out.

When aggregates are broken apart, the organic materials that were protected by being inside an aggregate are exposed to microbes, and then their decomposition generates pulses of carbon and nutrient mineralisation characteristic of the Birch effect (Fierer *et al*., 2003) [17]. The amount of slaking and aggregate breaking apart is dependent on how much moisture was present before the wetting occurred and how quickly the water was applied. For example, quickly applying water to an area that is severely dry will produce more aggregate breakdown than if done at a slower or hazard rate.

By contrast, moderate drying and re-wetting will allow for the stability of aggregates to develop over time due to the production of EPS, fungal hyphae, and glomalin-related soil proteins, all of which are produced by microbes, when aggregates are broken apart or have a high biological activity and/or more organic matter (Luo *et al*., 2013) [20]. Therefore, how aggregate stability is affected by AWD is very much determined by the situation; thus, future mechanistic research is warranted in the evaluation of groundnut production systems.

3.3. Soil Oxygen Dynamics During Wetting and Drying Cycles

One of the earliest and most dramatic changes observed in soil properties associated with air-water dynamics (AWD) is the availability of oxygen. After wetting of the soil, air-filled pores are replaced with water, and O2 depletion occurs after as little as hours for some microsites.

In heavy soils, those that have a high clay content, macro-anaerobiosis develops within the first 24-48 hours of saturation, leading to a predictable sequence of anaerobic respiratory processes (E.g., denitrification (NO3- to N2O and N2), manganese reduction, iron reduction, and ultimately, sulphate reduction). During AWD in upland ecosystems, the spatial extent of anaerobiosis is generally more localised than that in paddy soils, where anaerobic microsites are restricted to the interior of water-filled macroaggregates, while adjacent macropore-associated communities are restored to aerobic conditions.

This heterogeneity in the spatial availability of oxygen at the micrometre scale creates an incredibly diverse array of redox potentials across the small distances present in the rhizosphere, potentially supporting multiple aerobic and anaerobic metabolic processes and thus resulting in the high level of microbial diversity characteristic of rhizosphere environments. During the drying phase, O2 continuously infiltrates as the pores are drained and aerobic decomposer populations recover from dormancy or from residual populations that have survived in aerobic microsites. The rate and extent of aerobic recovery following drying is a significant factor determining the net biogeochemical effects of AWD cycles for C and N retention in soils.

3.4. Effects on Nutrient Availability and Transport Processes

AWD has a significant impact on the transport and availability of C, N, and P via several interacting pathways. The added physical disruption of aggregates creates increased availability of both direct organic carbon (C) through decomposition of physically protected organic matter and indirectly through the physiological death and autolysis of microorganisms that cannot survive rapid changes in osmotic pressure relative to drying. The dissolved organic carbon (DOC) released following the wetting event is a readily usable, labile carbon (C) pool that supports rapid microbial growth after re-wetting, thus priming a more recalcitrant soil organic matter decomposition as a function of the classical rhizosphere priming effect. This priming effect is particularly pronounced in Agave hypogaea due to both the relatively C-rich composition of the exudates produced by the legume and the high microbial biomass associated with the legume rhizosphere.

The means by which nitrogen becomes available in an AWD system is mostly determined by the processes of mineralisation, nitrification, denitrification and immobilisation. After rewetting events, a quick pulse of N mineralisation occurs from the microbial biomass and organic matter, and the resulting NH4+is then converted to NO3-under aerobic conditions via nitrification. However, if soil moisture remains high following a wetted condition, considerable amounts of the newly produced NO3-can be lost through denitrification and thus become a net loss of nitrogen in the system if managed appropriately with AWD. Phosphorus is similarly affected by iron; during the wetting process, reducing ferric iron releases Fe-bound phosphate into the soil solution, increasing P availability for a limited amount of time, and then subsequent oxidation of iron during the drying process causes P to become re-adsorbed into oxidised iron minerals (Cui *et al.*, 2011; Spohn and Kuzyakov, 2013) [21, 22].

3.5. Impacts on Microbial Habitat Conditions

AWD fundamentally transforms the spatial environment of the rhizosphere microorganisms in addition to impacting their nutrient supply by changing the amount of water available for them. For instance, the thickness of water films on soil particles, which has a significant influence on the continuity of the series of aqueous pathways (cell movement and the diffusion of substrates and exchange of metabolites) that exist for the microorganisms, drastically reduces as soil dries out (18). When the soil water potential drops to about -1.5 MPa (wilting point), the water films become discontinuous, and microorganisms are physically isolated, greatly decreasing the amounts of substrates available to the microorganisms and the rates at which any products are able to be removed from the environment surrounding the organisms. Organisms with the ability to create compatible solutes (such as betaine, ectoine, trehalose), thick cells walls, or form dormant structures (i.e., endospores or cysts) are greatly favoured in such conditions.

In addition, when water films are present during drying, the concentrations of solutes in the thin water films impose tremendous osmotic stress on non-adapted microorganisms, leading to membrane damage and cell death for sensitive taxa, while favouring the survival of osmotolerant taxa (19). The ecological impacts of this selective pressure are particularly apparent for the Gram-negative bacteria that dominate the rhizosphere under moist conditions: the Gram-negative bacteria have thinner peptidoglycan layers and greater membrane fluidity than the Gram-positive bacteria (Firmicutes and Actinobacteria), which makes them more susceptible to osmotic damage; therefore, a shift to greater dominance of the Gram-positive bacteria has been commonly noted when there is a drying event.

3.6. Long-term Ecological Consequences of AWD Practices

While short-term impacts of alternate wetting and drying conditions (AWD) on soil characteristics and microbial diversity are more well documented, long-term and cumulative impacts from repeating cycles of AWD over multiple cropping seasons are still only partially understood. Repeated AWD has the potential to progressively change the quality of soil organic matter by favouring mineralisation of labile (i.e. available for microbial decomposition) forms of carbon during each wet-to-dry cycle of each growth season, potentially causing the ratio of carbon to nitrogen (C:N) to increase for residual organic matter, and thereby, decrease its availability to the microbes that decompose organic matter (Allison *et al.*, 2010) [28]. This progressive humification could lead to reduced microbial biomass over time and a shift towards a microbial community more suited to the decomposition of low-quality organic matter on oligotrophic (i.e. low availability of nutrients) substrates.

On the other hand, improved soil structure from arbuscular mycorrhizal fungal (AMF) hyphal networks during dryer phases of growing seasons provides benefits from enhanced macroporosity due to root and earthworm activity, when aerobic conditions prevail, to improve the soil's long-term water-holding capacity and drought tolerance. The balance between the potential positive structural benefits and negative organic matter quality impacts of AWD will likely depend on: the particular soil type, how intensively AWD is managed, and whether or not there is a significant supply of organic matter through the return of crop residues. Therefore, long-term experimental work (> 3-5 years) is urgently needed to determine how these two competing dynamics interact.

4. Microbial Community Shifts Under Alternate Wetting and Drying Conditions

4.1. Changes in Bacterial and Fungal Diversity Under Moisture Fluctuations

Many studies have been conducted on how soil and rhizosphere microbial communities respond to changes in moisture by utilizing both culture-dependent and molecular methods, and these studies are presenting a growing body of evidence that suggests significant and consistent shifts in community composition, diversity, and functional activity occur at different periods of time in response to drying-rewetting cycles (15,17). For example, alpha-diversity indices (i.e., species richness and Shannon entropy) generally exhibit a curvilinear pattern with respect to soil moisture potential, with the largest value falling within the mid-range moisture content and decreasing at both extremes of absolute saturation and absolute desiccation (18). This pattern is consistent with the intermediate disturbance hypothesis, which posits that competitive exclusion occurs among specialist taxa under steady state (flood or drought), but there are many competing taxa that utilize various strategies in the temporally variable environment created by AWD.

Bacterial and fungal populations respond differently to humidity and will display different changes from one observation to another based on these differences which arise from the differences in composition of their cell wall, the differences in their ability to adapt to osmotic stress, and in the way that they grow and reproduce. Different groups of bacteria, especially the Proteobacteria and Firmicutes, are able to respond to rewetting much faster than fungi and will grow rapidly when water becomes available, reaching their maximum growth rates 24 – 72 hours after being rewet and taking advantage of the sudden influx into the soil of dissolved organic matter and mineral nutrients. Fungi, both arbuscular mycorrhizal fungi (AMF) and saprotrophic fungi, will grow slower than bacteria after being rewet due to their greater ability to tolerate desiccation stress due to their thick cell walls, hyphae that are divided into compartments, and their ability to synthesize osmolytes. As the ratio of fungal to bacterial biomass shifts during each alternate wetting and drying (AWD) cycle, it is an effective indicator of the entire community's response to moisture management.

4.2. Dominant Microbial Groups in Nutrient Cycling Under AWD

The major groups of microbes that take part in the nutrient cycles in agricultural soils, as well as their known roles, are listed in Table 3. The dominant microbial group in the groundnut rhizosphere is the phyla of Bacteria called Proteobacteria, which contain functionally diverse organisms associated with: (1) nitrogen fixation (Rhizobiales), (2) nitrification (Nitrosomonadales), (3) denitrification (Pseudomonadales, Rhodospirillales), (4) solubilising phosphorus (Burkholderiales), and (5) mineralising organic carbon. Rewetting the soil greatly increases their abundance as they have a reproductive strategy of growing rapidly on labile (easy to break down) substrates, which coincides with resource pulses associated with aggregate disruption (Bouskill *et al*., 2013) [15].

In contrast, the Actinobacteria (e.g., Streptomyces, Nocardioides, Micromonospora) are a K-strategist; they have a slow growth rate and have high drought tolerance through spore formation and the production of osmotic stabilisers. Actinobacterial abundance will increase in periods of drought when the abundance of Proteobacteria is decreasing, and they play key roles in the decomposition of hard-to-decompose plant material and the production of antibiotics that regulate the community structure of the larger microbial populations. While Acidobacteria are plentiful in normal soil, they are abundant in low nutrient soils; their numbers will increase when there is a lack of easy-to-use nutrients and a corresponding increase in soil pH (Kuramae *et al*., 2012) [25]. During the drying-rewetting cycle, there has been documented evidence for a marked increase in the measure of Firmicutes, especially for those endospore forming genera like Bacillus and Clostridium that respond very quickly to the availability of moisture, by germinating! The growth and recovery of Bacillus species, which can be found within the rhizosphere of groundnuts and act as documented plant growth promoting rhizobacteria (PGGR) or phosphorous solubilisers, may indicate to a certain extent their continued functional resilience in their capacity to mobilise phosphorous (P) Sources through regular wetting/drying events occurring through the Alternate Wet/Dry (AWD) cycles (Vessey, 2003; Glick, 2012) [8].

Table 2: Physicochemical changes in soils under alternate wetting and drying conditions.

Soil Property	Response to AWD	Underlying Mechanism	Consequence for Nutrient Cycling
Soil pH	Slight decrease during wetting; rebound during drying	Redox-driven proton release; organic acid accumulation	Affects nutrient solubility and microbial enzyme activity
Aggregate stability	Initial disruption during rapid wetting; recovery with drying cycles	Slaking forces from rapid water entry; fungal hyphal binding during drying	Macroporosity, water infiltration, and gas exchange
Bulk density	Transient reduction post-wetting; compaction risk at field capacity	Swelling clays and bioturbation; heavy machinery during moist periods	Root penetration resistance and waterlogging risk
Water-holding capacity	Temporarily increased by organic matter accumulation	Microbial biomass and EPS production enhance aggregate water retention	Drought resilience and nutrient transport
Cation exchange capacity (CEC)	Modest increase with repeated AWD cycles	Enhanced organic matter and clay mineral interactions; H^+ displacement during drying	Nutrient retention and buffering capacity
Redox potential (Eh)	Sharp decline during saturation (-200 to -300 mV); recovery on drying (+400 to +600 mV)	Electron acceptor depletion sequence: O_2, NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-}	Controls denitrification, Fe/Mn solubility, and P availability
Dissolved organic carbon (DOC)	Spike upon rewetting; rapid decline with drying	Cell lysis and aggregate disruption release soluble C; rapid microbial consumption	Priming effect and C mineralization pulses

Table 3: Major microbial taxa and their functional roles in nutrient cycling in groundnut rhizosphere soil under AWD conditions

Microbial Taxa	Physiological Type	Key Functional Roles	Response to AWD Moisture Regime
Proteobacteria (Alpha, Beta, Gamma)	Highly diverse; aerobic and facultative anaerobic	N fixation (Rhizobium, Bradyrhizobium), nitrification (Nitrosomonas), P solubilization, C mineralization	Dominant in wet, nutrient-rich rhizosphere; proliferate upon rewetting
Actinobacteria	Aerobic; spore-forming; filamentous	Lignocellulose decomposition; antibiotic production; organic N mineralization; humus formation	Increase under drying; key in recalcitrant C degradation
Acidobacteria	Predominantly oligotrophic; acidic soils	Polysaccharide degradation; iron reduction under anoxia; slow C cycling	Sensitive to nutrient pulses; decline in copiotrophic conditions
Firmicutes	Endospore-forming; anaerobic and aerobic	Fermentation; denitrification; N fixation in some genera; stress tolerance	Resurgent after drying-rewetting cycles; anaerobic metabolisers
Verrucomicrobia	Aerobic; soil-specific	Degradation of complex polysaccharides; contribution to C cycling	Relatively stable under moisture fluctuations
Arbuscular mycorrhizal fungi (AMF)	Obligate symbiont	P acquisition via extensive hyphal networks; water uptake; C allocation to soil	Enhanced P uptake efficiency; sensitive to repeated desiccation
Saprophytic fungi (Trichoderma, Aspergillus)	Aerobic saprotrophs	Lignocellulose decomposition; P solubilization via organic acid exudation; biocontrol	Dominant during aerobic drying phases; reduced under anaerobiosis
Methanogenic archaea	Strictly anaerobic	CH4 production from CO2/H2 and acetate during prolonged saturation	Relevant in AWD rice systems; minimal in well-drained groundnut soils

4.3. Community Succession and Adaptation Mechanisms

In general, community succession in an AWD ecosystem has predictable trajectories, reflecting the different ecological life-histories of the most abundant taxa, as well as the order in which resources become available during the wet-dry cycle. A copiotrophic explosion occurs immediately after rewetting, with the largest increases in abundance being observed among the fastest growing Gammaproteobacteria and Betaproteobacteria which utilise an increase in dissolved organic carbon resulting from aggregate disruption and the release of large amounts of microbial necromass prior to and during the wet-dry cycle (Fierer *et al*., 2003) [17]. Following the copiotrophic explosion, communities transition to a slower-growing oligotrophic state as the labile available substrates are exhausted, a period of time that will vary from approximately 5-15 days depending on the relative magnitude of the initial substrate pulse to and the rate of continuous exudate production from plant roots (Bouskill *et al*., 2013) [15].

There are many ways that an organism can get adapted to air-to-water ratios. There are many different time frames over which to measure these changes in an organism's physiology. An organism can change the way it uses osmolytes to adapt to moisture fluctuations by changing the way that it conserves energy and water through the composition of its membrane fatty acids, and by altering the way that it regulates the gene expression of stress-response genes through transcriptional regulation (Kaisermann *et al*., 2017) [19]. In addition, a population of an organism will adapt to increases or decreases in air-to-water ratios, which will lead to changes in the frequency of alleles (or variants of genes) in the population over a series of wet-dry cycles. Finally, over multiple wet-dry cycles, the composition of the microbial community will change due to changes in the relative abundance of stress-tolerant and other types of microbes. This ongoing succession towards stress tolerance, directly and indirectly, helps establish the memory or legacy effect of the changes made by the previous moisture management practices (Kaisermann *et al*., 2017) [19]. These results have important practical applications regarding the management of air-to-water ratios. The composition and level of resilience exhibited by the microbial community at the start of the wet season will be a reflection of the cumulative legacy of the moisture management practices employed by the land manager prior to the start of the wet season.

4.4. Functional Redundancy and Resilience

The ability for different kinds of organisms to do the same thing is called functional redundancy; this attribute is a very important thing for microorganisms living in the soil because it allows the soil ecosystem to be able to maintain its function after it has been disturbed (e.g., fluctuating moisture levels). Under alternating wet and dry conditions, functional redundancy will help to ensure that critical biogeochemical processes (e.g., nitrogen (N) mineralisation, nitrification, and phosphorus (P) solubilisation) continue even though there are (significant) changes in the community of microorganisms during moisture cycles because there are multiple types of microorganisms that can do the same thing, just like there are many different people doing the same job. There are studies that have found that the rates of processes (e.g., CO2 efflux, N2O emission and enzyme activity) are restored much faster than the microorganisms that contributed to those processes; therefore, although the types of microorganisms (i.e., the community composition) may be different following a moisture disturbance, the types of work accomplished will be the same (Hartmann *et al*., 2015) [16]. However, the level of functional redundancy is neither indefinite nor universal. Some specialised functions performed by narrow groups of members of the same taxonomic group have very little functional redundancy, including: biological nitrogen (N2) fixation by symbiotic organisms with legumes, methane oxidation, and the production of certain secondary metabolites (e.g., flavonoids). The specialised functions that do functionally lack redundancy will be the most at risk for perturbation; thus, these specialised functions will greatly impact the N nutrition of plants and the health of plants relative to the very minimal impact that they will have on the larger, general processes of C and P mineralisation that are well protected by functional redundancy (Salvagiotti *et al*., 2008) [12].

4.5. Microbial Interaction Networks Under Changing Environmental Conditions

The moisture levels being impacted through alternate wetting and drying (AWD) caused substantial reorganization to the ecological network of interactions within rhizosphere microbial taxa (including mutualism, competitive, parasitism, and cross-feeding), whereas network analysis methods have identified that microbial co-occurrence networks become less connected and more modular than in dry conditions due to stress (e.g., drought conditions). There is a decrease in positive interactions and an end to cross-feeding relationships, thus breaking apart a community into independently functioning modules as a result of resource limitation. However, rewetting can create temporary increases in network connection as rapidly growing copiotrophic taxa produce metabolic by-products that support secondary consumers, thus establishing new, short-term (short-lived) interaction networks based on the pulse of organic matter created.

Network-level dynamics may provide some implications for ecosystem functions that cannot be derived from studies at both the individual taxon and functional group levels alone. Drought-affected, highly modular networks may sustain functional capacities at the modular level, yet lose cross-modular interactions that typically connect C and N cycling pathways. For example, decoupling of Bradyrhizobium N-fixing from P-cycling community during times of extended drought may lead to less efficient fixed N delivery via P-mobilized pathways, even though both 'functional groups' maintain independent functional capacities.

Measurement of network-level effects through structural equation modelling (SEM) and multilevel network analyses (MLNA) are very powerful statistical tools that are not yet being utilized to their full potential in AWD-based rhizosphere research.

5. Coupling Between C:N:P Stoichiometry and Microbial Function

5.1. Relationships Between Nutrient Ratios and Microbial Metabolism

A core area of modern soil ecology has been the degree to which microbial metabolism relates to substrate stoichiometry, and there is strong evidence that microbial community composition, growth efficiency, and the net direction of nutrient transformations are determined, to some extent, by the ratio of carbon (C), nitrogen (N), and phosphorus (P) in the available substrates. Growth efficiencies are maximized and are tightly linked to nutrient cycling when the C:N:P ratios of the substrates are closely aligned with the stoichiometry of microbial biomass, which has been estimated to be approximately 60:7:1 (Cleveland and Liptzin, 2007) [2] (Xu *et al.*, 2013) [4]. On the other hand, if substrates have a high C:N:P ratio (high C relative to N and P), then microorganisms have two options: they can either immobilize N and P from inorganic sources and use them to synthesize biomass or reduce their growth efficiencies by mineralizing the excess C into CO2 through increased respiration.

In the rhizosphere of A. hypogaea, AWD-driven changes in the quality and availability of substrates lead to large alterations in the stoichiometric landscape of the microbial community. The rewetting pulse presents a mixture of substrates with different stoichiometries, with labile (easily decomposed) and C-rich substrates from aggregate fragmentation contributing predominantly to a higher C:N:P ratio, and necromass (i.e., dead microbial biomass) contributing to a relatively lower C:N ratio, combined to form a more balanced substrate. The overall stoichiometric effect of the rewetting pulse and the associated timing of contributions from these different substrates will, therefore, need to be considered together when predicting microbial community responses from relatively simple measurements of substrate chemistry.

5.2. Effects of Stoichiometric Imbalance on Microbial Growth and Activity

When there is an imbalance in the ratios of carbon and nutrients in the environment, this creates a metabolic cost to the microorganism and must be compensated through changes in the acquisition of resources and the allocation of those resources. When microorganisms are limited by carbon (low carbon-to-nutrient ratio), they decrease the amount of resources that are utilized to produce their biosynthetic related macromolecules (i.e. proteins and nucleic acids) and increase the amount of resources allocated toward producing energy generating catabolic enzyme activity, often resulting in an increased soil enzyme activity per unit of microbial biomass (Zeng *et al.*, 2017) [5]. One example of this is observed in the rhizosphere of A. hypogaea during the dry phase of the alternating wet and dry (AWD) cycles, when root exudate inputs are reduced, and thus the microorganisms in the rhizosphere are forced to rely on the recalcitrant organic matter in the soil as their primary substrate.

When microorganisms are limited by either nitrogen or phosphorus (high carbon-to-nitrogen or carbon-to-phosphorus ratio), they face capacity to produce the protein-rich cellular machinery that is required for rapid growth (i.e. biosynthetic constraint). In response to nutrient limitation, microorganisms produce more extracellular enzymes that can be used to acquire nitrogen and phosphorus from the soil (i.e. urease, protease, and phosphatase) and reduce the carbon use efficiency for their metabolic processes, thereby directing more of their substrate carbon toward producing CO2 via increased respiration instead of biomass (Allison *et al.*, 2010) [28]. The relationship between stoichiometric imbalances and reduced carbon use efficiency will also affect the dynamics of soil organic matter and may contribute to greenhouse gas emissions, as a high amount of carbon that is transferred to the rhizosphere from plants will be respired instead of stored in microbial biomass and later stored as mineral-associated organic matter.

5.3. Microbial Carbon-Use Efficiency and Nutrient Allocation Trade-offs

The microbial carbon use efficiency (CUE), which defines how much of the carbon taken up by the microbes is used to create biomass versus being lost via respiration/rate of growth, is an important driver of organic carbon accumulation in the soil and how much carbon is retained in the soil over the long-term39. Substrate stoichiometry strongly affects CUE - when the ratio of carbon to nitrogen to phosphorus (C:N:P) of the substrate is close to what the microbial community needs, CUE will be high (0.4-0.6), but when it is out of balance with respect to the microbial community needs, it will take metabolic energy from carbon (and therefore reduce the amount of carbon used for biomass growth) and divert it to maintain homeostasis of nutrients in the cell. The temporary stoichiometric imbalance of substrates due to the moisture driven shift in substrate quality is believed to lead to lower average CUE during the growing season in AWD systems compared to continuously wet or dry soils, with subsequent impacts of soil C stocks over time.

CUE measurements obtained through empirical methods in AWD soils are limited at this time, therefore making estimates of CUE from stoichiometric modelling different from CUE calculated through direct measurement with isotopically-labelled substrates39. To solve this discrepancy, careful consideration needs to be given to the timeframes CUE is estimated and how they correspond with moisture pulses and the turnover rates of microbial biomass. Additionally, methodological challenges such as substrates differing from the microbially-available (or from the perspective of the microbial community), wet-dry cycle information, dormancy-active transitions, as well as changes in community composition can significantly affect CUE estimates, which can limit our ability to predict quantitatively how AWD will affect overall soil C balance.

5.4. Enzymatic Regulation Associated with Nutrient Acquisition

Extracellular enzymes in soil show the microbial allocation of resources to obtain external nutrients, while their stoichiometry reflects the relative demands for nutrients among the viable microorganisms present in a community (Zeng *et al*., 2017) [5]. Table 4 shows the main enzymes that are involved in the acquisition of C, N and P with their corresponding reactions and the effect of alternating wetting and drying (AWD) on them. Beta-glucosidase (BG), which catalyzes the last step of cellulose hydrolysis, provides an indication of the demand for acquisition of carbon (C). Urease, which catalyzes the hydrolysis of urease to NH4+, and leucine aminopeptidase(LAP), which cleaves peptide bonds, provide an indication of the capacity of microorganisms to acquire nitrogen (N). Acid and alkaline phosphatases catalyze the hydrolysis of organic phosphate esters and provide an overall estimate of the demand for phosphorus acquisition.

In the rhizosphere, the relationship of BG to (NAG+LAP) and BG to phosphatase activity provides an ecoenzymatic stoichiometric index for diagnosing relative limitations (C, N or P) to plant growth. Under alternating wetting/drying (AWD) conditions, the BG:NAG+LAP and BG:phosphatase activity ratios change with moisture: during the wet phase C-limiting indicators (high BG compared to N-acquiring and P-acquiring enzymes) are typically observed when N and P are released from the breakdown of organic matter and the decomposition of microbial biomass; during the dry phase, P- and N-limiting indicators appear as C inputs from root exudation decrease. Dehydrogenase activity, used to measure the overall electron transport activity of the microbial community, represents an integrated measure of total microbial metabolic activity and is one of the most sensitive enzymatic indicators of the impact of AWD moisture management practices.

Table 4: Enzymes involved in microbial nutrient acquisition and biogeochemical processes in rhizosphere soils.

Enzyme	Nutrient Cycle	Reaction Catalysed	Response to AWD Conditions
beta-Glucosidase (BG)	C cycling	Cellobiose --> glucose (final step of cellulose hydrolysis)	Increases with rising soil moisture; inhibited by prolonged anaerobiosis; used as C-limitation indicator
N-acetyl-glucosaminidase (NAG)	N and C cycling	Chitin degradation releasing glucosamine; amino sugar recycling	Correlated with fungal biomass; decreases during drying phases
Leucine aminopeptidase (LAP)	N cycling	Peptide bond hydrolysis releasing amino acids for microbial N acquisition	Increases post-rewetting when labile N substrates accumulate
Urease	N cycling	Urea --> NH4+ + CO2; mineralizes organic N	Activity stimulated by warming and moderate moisture; inhibited by desiccation
Acid/alkaline phosphatase	P cycling	Organic P ester hydrolysis releasing inorganic phosphate	Inversely correlated with inorganic P availability; increases under P limitation in AWD systems
Phytase	P cycling	Inositol hexaphosphate (phytate) hydrolysis; major organic P substrate	Enhanced by root exudates of groundnut under P deficiency
Dehydrogenase	Electron transport / redox	Overall oxidative microbial metabolism; electron transfer in ETS	Sensitive indicator of microbial metabolic activity; declines with desiccation; recovers rapidly on rewetting
Arylsulfatase	S cycling	Sulfate ester hydrolysis; organic S mineralization	Useful complementary enzyme in multi-nutrient stoichiometric analyses

5.5. Nutrient Constraints Affecting Microbial Functioning

The interaction between constraints to nutrient availability and microbial functions in the AWD managed groundnut rhizosphere shows complex feedback loops that are inadequately represented by traditional single limit frameworks for nutrient availability. Current multi-nutrient limitation theories suggest that C, N and P can co-limit the growth of microbes, especially with the stoichiometric variability inherent in moisture fluctuation conditions (Elser *et al*., 2007) [1] (Vitousek *et al*., 2010) [31]. The high prevalence of states of co-limitation are significant because they can prevent the complete recovery of microbial biomass and function following perturbations to moisture content: for example, a microbial community recovering from drying stress will potentially have an excess of C following rewetting but will also be unable to use all of its available C because the microbes have also been made P limited through the irreversible fixation of P to re-oxidised iron minerals (Cui *et al*., 2011) [21].

Multi-nutrient limitations have a direct effect on how we interpret the impact of AWD on crop productivity through rhizosphere microbial communities. If N and P are limited at the same time for microbial communities in certain phases of the AWD cycle, then the ability of these microbes to provide nutrient services to the crop might be periodically restricted, even if the total soil nutrient pools look sufficient based on bulk chemical analysis. Therefore, to achieve additional mechanistic understanding of how AWD affects rhizosphere function, it is necessary to measure multiple nutrient forms in both time and space, as well as measure microbial biomass and activity data together with nutrient measurements.

6. Rhizosphere Biogeochemical Processes and Nutrient Cycling

6.1. Carbon Mineralization and Stabilization Pathways

In the A. hypogaea rhizosphere, carbon cycling consists of many complex mineralisation and stabilisation activities which are regulated differently by moisture regime, substrate quality, and community composition. Mineralisation is the process of converting organic carbon to CO2 or CH4 and is carried out by heterogeneous groups of bacteria and fungi. These bacteria and fungi produce many different types of extracellular enzymes that degrade polysaccharides, proteins, and lignin to provide the substrates needed for mineralisation. When soil is kept under alternating wetting and drying conditions, mineralisation occurs via pulses rather than continuously. Each time soil is rewetted, it results in a burst of CO2 being released into the atmosphere (the Birch effect) that can be 2-10 times greater than what would normally occur in soil that is kept continuously moist (Fierer *et al*., 2003) [17].

Drying phases associated with alternate wetting and drying (AWD) systems lead to increased carbon (C) stabilisation by converting microbial processed C into forms that are mineral-associated or physically protected in aggregates with long mean residence times (i.e., several years). Furthermore, dissolved organic C can be concentrated on and adsorbed to mineral surfaces, as well as physically encapsulated within desiccated aggregates during the drying phases of AWD. The clay mineralogy of a given soil type is one of many critical determinants of the balance between mineralisation (i.e., breakdown of organic matter by microbes) and stabilisation (i.e., the formation of new soil organic matter (SOM) after C has been mineralized). High-activity clays (i.e., smectites) have a much higher stabilisation capacity than low-activity clays (i.e., kaolinite) that predominate in tropical Alfisols of groundnut-growing regions. Thus, the overall effect of AWD on soil C storage will depend on the relative contribution of enhanced mineralisation during times of rewetting versus enhanced stabilisation during periods of drying, and this effect could vary significantly depending on the management intensity and soil type.

6.2. Nitrogen Transformation Processes Under AWD

N transformations in the groundnut rhizosphere under aeroponic systems include all areas of N cycling, including fixation, mineralisation, nitrification, denitrification, and assimilatory/dissimilatory nitrate reduction. The principal input pathway in well-managed groundnut systems is biological N fixation by Bradyrhizobium in nodules, along with additional asymbiotic N fixation performed by free-living diazotrophic bacteria such as Azospirillum and Gluconacetobacter The symbiotic and asymbiotic N fixation processes are both affected by moisture levels in the soil; excessive moisture can limit O2 availability, while extreme drought conditions will result in limited carbon availability or osmotic stress affecting the metabolism of bacteria living in the nodules.

When there is oxygen present, nitrosomonas, nitrobacter, and ammonia oxidizing archaea (AOA) carry out the conversion of ammonium (NH4+) to nitrate (NO3-) in a process called nitrification. Nitrification occurs in the aerobic and dry phase of alternate wetting and drying (AWD) but may be inhibited after the rewetting event due to short-lived anaerobic conditions created during the drying phase. During the wet phase, denitrifying bacteria use nitrate (NO3-) and convert it to nitrous oxide (N2O) and dinitrogen (N2), creating anaerobic microsites to facilitate this process around larger, water-filled macroaggregates. Therefore, the net affect of AWD on the nitrogen balance of the soil will depend on how long aerobically and how much time spends in anaerobic condition, how quickly the plant can use the mineralised nitrogen before it is lost via denitrification or leaching and how much nitrate and easily decomposable carbon is available for denitrification as an electron donor.

6.3. Phosphorus Solubilization and Mobilization

Crop nutrition from phosphorus cycling in tropical soils has specific difficulties because inorganic phosphates are held by iron and aluminium oxides and only a portion of all P that exists in organic ester forms is available to plants until prior enzymatic hydrolysis (Walker and Syers, 1976) [32]. Alternate wetting and drying creates dynamic changes in the availability of P due to many mechanisms such as: the dissolution of iron minerals during the wet/anaerobic phase results in phosphates that were bound to iron being available (Fe bound) for use, thus creating a potential large pulse of available P for plants; and during the wetting of aggregates, there is disruption of aggregates, thereby exposing organic and mineral forms that were occluded prior to wetting; and when microorganisms die due to drying out (osmotic stress) and subsequent rapid rewetting, the microorganisms release the phosphates that were inside their cells as they had access to too much P between wetting and drying (Cui *et al*., 2011; Spohn and Kuzyakov, 2013) [21, 22].

Microorganisms (PSM) that solubilize phosphate (including Bacilus, Pseudomonas & Aspergillus) produce organic acids (e.g., geo-conic, citric, oxalic) that help to decrease the pH in the rhizosphere, thereby removing phosphate from the mineral-bound sites. The activity of PSM is influenced by the moisture content in the soil. Intermediate moisture levels in the soil provide optimal conditions for the production and diffusion of the organic acids to the mineral surfaces, whereas excessive drought or saturated moisture levels will restrict PSM activity, and thus decrease the rate of P solubilization. One of the important interactions for contributing to the maximum efficiency of N and P acquisition by the plant in the groundnut rhizosphere is the temporal relationship between N fixation (Bradyrhizobium mediated) and P solubilization (PSM mediated) in the groundnut rhizosphere; therefore, changes to the timing and soil moisture management of AWD systems have the potential to significantly disrupt this interaction, which is an unrealized method by which moisture management affects the nutritional status of groundnut.

6.4. Rhizosphere Priming Effects

The purpose of this paper is to describe the process of how roots affect the decomposition of native soil organic material in the rhizosphere of Arachis hypogea. Priming occurs when the addition of carbon (C) from the root system accelerates the decomposition of previously existing, stable soil organic materials. Due to the large amounts of C provided from root exudates, it is likely that priming of soil organic matter by A. hypogaea roots will be very high as a result of two constraints: (1) constant input of new C from root exudates and; (2) a nitrogen (N) deficit from lack of biological nitrogen fixation (BNF). Priming is likely to be influenced by soil moisture and will vary in intensity throughout an irrigation cycle. In the case of alternate wetting and drying (AWD), C from root exudates will be delivered to the soil in pulse-like fashion during wet atmospheric conditions and withheld from the soil in gradually declining amounts during dry atmospheric conditions. As such, the intensity of priming during AWD should be greater in relation to time than in continuously wet soils.

Priming effects have a unique magnitude and direction determined by the stoichiometric quality of a priming substrate in relation to the original organically bound materials from which it decomposes. The C:N ratio of a soil has a primary influence on microbial community structure and the location of growth; specifically, if an exudate C-N ratio is low (i.e. N- rich exudates during pod fill), then priming will likely shift to negative as the microbial community changes from co-decomposing stable organic matter to directly taking up the high quality exudate C. These stoichiometric controls on priming directionality will likely provide for an important area of future research on C cycling in shrub communities under different moisture regimes.

6.5. Interactions Among Nutrient Cycling Pathways and Feedback Mechanisms

Groundnut rhizosphere cycling of C, N and P are interrelated via biogeochemical feedbacks that either enhance or degrade the multiplicative influence of AWW on C, N and P cycles. C and N mineralisation ratio is stoichiometric, so when C is mineralised during wetting, there is a concomitant N mineralised, resulting in a pulse of N availability, which subsequently stimulates activity of nitrifiers and plant uptake (Zeng *et al*., 2017; Brady and Weil, 2016) [5, 11]. Phosphorus availability has feedback to CUE by altering microbial CUE; when P is limiting, it reduces CUE of microbes, therefore increasing the C:P ratio of microbial biomass turnover products, thus allowing for a greater amount of C to be released per unit of P cycled and potentially intensifying soil C loss under P-limited AWW conditions.

Plant-microbe interactions provide yet another aspect of interaction among these components, where increased plant growth and exudate production (due to increased nitrogen after a rewetting event) contribute to additional microbial growth and enzymatic activity, forming a positive system feedback loop that promotes rapid cycling of nutrients for weeks following reirrigation. On the other hand, nutrient loss through denitrification during extended wet/dry periods or leaching during reirrigation is viewed as creating negative system feedback on plant growth and exudate production, reducing the rhizosphere priming effect and consequently limiting the rate of nutrient cycling. Therefore, understanding and managing the feedback relationships associated with these plant-microbe systems is essential for optimising AWD management of groundnut for productivity and soil health.

7. Soil Physicochemical Responses and Crop Performance

7.1. Effects of AWD on Soil Physicochemical Properties

AWD management creates an environment for soil and other materials that directly impacts the availability of microorganisms in the rhizosphere and also affects the physical conditions experienced by groundnut roots. Physically and chemically, the soil's pH is one of the most important factors affecting nutrient availability, microbial diversity, and the activity of various soils enzymes; but the effect of AWD on pH is complex and is often dependent on interactions between the soil's ability to acidify (via organic acid production, CO2 production through flooding, and nitrification during drying down) and alkalinize (via proton consumption during denitrification and iron reduction); (Brady and Weil, 2016) [11] thus, in the tropical Alfisols and Ultisols that are typically found in groundnut-producing regions, temporary acidification caused by AWD during the wetting phase may enhance the solubility of phosphorous from calcareous patches, increase the activity of acid phosphatase and phosphorus solubilizing microorganisms (Pseudomonas) during the wetting phase.

The soil's ability to resist pressure from plant roots and provide habitat for microbes is determined in part by the stability of its aggregates. Aggregate stability can be affected by AWD frequency and intensity, and follows a nonlinear response. Moderate frequencies and intensities of AWD that do not allow complete drying can help improve aggregate stability through increased deposition of microbial polysaccharides and deposits of AMF glomalin in the macroaggregates; whereas frequent and/or severe cycles of drying and wetting will lead to a net disruption of aggregates due to slaking force (Luo *et al*., 2013) [20]. These results have significant implications for groundnut production because pod formation in the soil requires sufficient macroporosity to allow expansion during fill and adequate gas exchange in the peg zone. Therefore, very compacted or structurally degraded soils can limit pod fill regardless of available moisture or nutrient supply in the root zone.

7.2. Effects on Nutrient Availability and Microbial Habitats

The two most agriculturally relevant physicochemical properties affected by AWD, via its influence on soil organic matter and biological activity, are water-holding capacity (WHC) and cation exchange capacity (CEC); WHC increases with increasing organic matter content; therefore, the overall impact of AWD on WHC will depend on which will dominate (mineralisation vs stabilisation) when looked at via the current methods of soil management and type of soil. Higher WHC provides more resilience to fluctuations in moisture, thereby lessening the severity of osmotic stress experienced by the root zone microbial community and resulting in less extreme physiological and chemical disturbance over the course of subsequent AWD cycles. This WHC buffering exerts a positive influence on the relationship between organic matter management, AWD resilience and stability of the microbial community.

CEC determines the ability of the soil to hold onto cationic nutrients (NH4+, K+, Ca2+, Mg2+) against leaching losses during irrigation events, thus its ability to maintain or gain CEC under AWD is of particular relevance to nutrient-use efficiency. Organic additions to the soil result in an increase in CEC via the functional groups of humic substances therefore will partially offset any loss in CEC that may occur should AWD cause organic matter mineralisation net. Integrating organic matter management with AWD water management is therefore a holistic approach for maintaining both the physical and chemical fertility of soils used for groundnut production systems.

7.3. Impacts on Groundnut Productivity

The combined effects of Automated Wetland Draining (AWD) on physicochemical properties, stoichiometric relationships, and the functional activity of microbial communities in the rhizosphere will ultimately impact the growth, development of pods, and yield of Arachis hypogaea. Root expansion is especially influenced by AWD management; for example, adequate moisture during early vegetative growth is necessary for lateral roots to develop and for nodules to form, and dry periods following this growth may result in an increased rate of root growth and deeper root systems as the plants respond to decreased moisture in the topsoil. When AWD is correctly managed, depth and breadth of the root system will be increased, which may improve the root system's access to sub-surface moisture and nutrients, particularly when surface soils are dry (Marschner, 2012) [6].

The yield response of A. hypogaea pods to AWD has considerable variation as evidenced in the literature. Pod yields have been reported to remain the same or increase with mild, well-managed AWD, while pod yields have been reduced with poor management of severe AWD. Critical factors influencing pod yield responses to AWD are the timing of moisture stress occurrence relative to pod initiation and filling (the periods most sensitive to moisture stress), the level of drying between irrigation events, and the soil's ability to provide nutrients during periods of moisture stress. In the presence of moderate AWD, nutrient uptake efficiency (specifically N and P) may be improved through temporary solubilized P pulses after rain and increased P availability through the stimulation of mycorrhizal networks during the drying period, resulting in greater P acquisition, on a per unit of root mass basis, in plants experiencing moderate AWD.

7.4. Implications for Agricultural Sustainability

The implications of AWD management for sustainability in groundnut production go far beyond yield metrics to include the efficiency of water use, greenhouse gas emissions, temporary soil health trajectories, and long-term land use productivity. Water use efficiency is increased for AWD as compared to conventional full irrigation, with documented savings of 20% to 40% in humid tropical groundnut systems while still producing acceptable yield levels [6]. These water savings have an immediate economic value in low water availability environments and increase water security regionally by decreasing competition between agricultural water needs and those for domestic and industrial purposes.

The greenhouse gas implications of AWD with groundnuts have the potential to be favourable when compared with both continuous flooding and severe drought management. Moderate AWD systems can consequently limit the N2O produced from denitrification, since prolonged anaerobiosis would be avoided; the same does not apply to flooded rice paddies where there is virtually unlimited CH4 production. Thus, the net total greenhouse gas emissions from AWD-managed groundnut systems would appear to favourably reflect on climate change, though a comprehensive lifecycle analysis that considers CO2, N2O, and other indirect greenhouse gas emissions associated with the infrastructure being used for water supply and energy inputs has yet to be published.

8. Comparative Analysis of AWD Treatments on Microbial and Nutrient Dynamics

The findings associated with a thorough comparison of the effects of different methods of controlling moisture in relation to rhizosphere microbial communities and nutrient cycling processes are essential to understanding the ecological effects of AWD methods of management. Table 5 includes a summary of the major biological and chemical indicators for each of the three management techniques: Continuous Flood (High Moisture), Continuous Dry (Drought), and AWD. The results of the study show that AWD creates a dynamic and intermediate ecosystem that takes advantage of resource pulses that are not accessible to either the continuously wet or continuously dry ecosystem. This results in higher peak values for many of the indicators of biological activity, but also may increase both the variability and temporal unpredictability in the process of supplying and cycling nutrients.

Table 5: Comparative impacts of AWD treatments on microbial communities and nutrient dynamics relative to continuous moisture management strategies.

Parameter	Continuous Flooding / High Moisture	Continuous Drying / Drought Stress	Alternate Wetting and Drying (AWD)
Microbial biomass carbon (MBC)	Moderate increase (+15-30%)	Decline (-10-20%)	Pulse (+40-60%) followed by gradual stabilisation
Bacterial alpha-diversity (Shannon index)	Moderate increase	Decrease with prolonged drought	Significant increase; r-strategist proliferation
Fungal:bacterial ratio	Decrease (bacteria dominate)	Increase (fungi more desiccation-tolerant)	Transient bacterial peak; recovery of fungi at intermediate moisture
Proteobacteria relative abundance	High (+)	Moderate decrease	Highest abundance; rapid response to C and N pulses
Actinobacteria relative abundance	Moderate	Highest relative abundance	Decrease immediately post-rewetting
Soil N mineralisation rate (mg N kg-1 d-1)	0.8-1.5 (aerobic)	0.2-0.5 (limited by moisture)	2.5-4.0 (Birch effect peak)
Denitrification losses (kg N ha-1 season-1)	Minimal (<0.5)	Negligible	Moderate (1-5 depending on NO3- availability)
Phosphatase activity (nmol g-1 h-1)	Moderate increase	Decrease with desiccation	Sharp increase; P solubilisation pulse
beta-Glucosidase activity (nmol g-1 h-1)	Highest under optimal moisture	Significant reduction	Peak within 24-48 h of rewetting
Available soil P (mg P kg-1)	Reduced (P immobilisation)	Variable; mineralisation from dead biomass	Significant increase due to aggregate disruption and microbial lysis

When interpreting these comparisons, consideration must be given to how time affects the results. Peak levels of many measures reported (microbial biomass carbon pulse after rewetting; N mineralisation rate from the Birch effect; phosphatase activity) represent a "better" level of performance, but possibly not over the entire growing season. The variation that occurs with AWD managed systems and the time differences between the nutrient release pulses and the peak demand by the crop are the major limitations agronomically of this management strategy and need attention through precision irrigation scheduling and complementary fertility management methods.

9. Emerging Approaches and Future Perspectives

9.1. Multi-Omics Approaches for Rhizosphere Characterisation

High-throughput sequencing technology has led to a paradigm shift in our understanding of rhizosphere microbiology and allowed us to understand how microbial community structure is related to microbial activity at resolutions never before possible. The molecular tools used to define the composition of prokaryotic (bacteria and archaea) and eukaryotic (fungi) microbial communities by PCR amplicon analysis (e.g., 16S rRNA for bacteria and archaea; ITS for fungus) have become commonplace in studying the impacts of AWD on the diversity and structure of microbial communities (Griffiths *et al*., 2000) [23] (Muyzer and Smalla, 1998) [26]. Unfortunately, these molecular methods can only provide information about the composition of the microbial community and cannot distinguish between active and dormant (non-active) cells. Furthermore, the diversity of microorganisms identified through PCR amplicon analysis contains many organisms that are not currently contributing to ecosystem function due to their dormant state at the time of sampling.

Through the use of total soil DNA through shotgun sequencing (Metagenomics), researchers can access the functional gene collections of all organisms in the soil to reconstruct the specific metabolic pathways for organisms in the rhizosphere. This includes the metabolic pathways responsible for biological nitrogen fixation (nifH), nitrification (amoA), denitrification (nosZ, nirK), and phosphorus (P) solubilization in the soil (pqq) (Griffiths *et al*., 2000) [23]. However, while sequencing total soil DNA will provide information on the potential metabolic pathways present, sequencing total soil RNA using metatranscriptomics will provide insight into what pathways are currently being expressed during sampling, and connect community composition and present metabolic activity more directly. Therefore, metagenomic and metatranscriptomic analyses of microbial communities in agricultural ecosystems and studies conducted with alternate wetting and drying (AWD) irrigation practices will help determine the main functional transitions occurring over the moisture cycles and provide information needed to identify resilient to vulnerable functional capacities.

9.2. Stable Isotope Probing and Advanced Biomarker Approaches

SIP methods allow the identification, at the level of individual taxa, of the living organisms that participate in biogeochemical transformations in the rhizosphere by monitoring how much of a substrate containing a stable isotopic label (13C-plant exudates, 15N-ammonium, 33P-phosphate) is incorporated into microbial biomarkers, including DNA, RNA, PLFAs, and amino acids. SIP can be applied to understand which bacterial and fungal taxa of AWD rhizospheres are utilising labile C and N released from the Birch effect, which are incorporating fresh fixed N from nodule turnover, and which are responsible for mineral P solubilisation. This ability to mechanistically elucidate the roles of different taxa is currently lacking in the majority of AWD research, which uses correlations between community composition and bulk biogeochemical data for inference.

Phospholipid fatty acid analysis (PLFA) is a method to measure how many microbes there are and what kind of metabolism those microbes have without having to grow them in a lab. Measuring PLFAs allows us to estimate the amount of metabolically active living microbes much more accurately than either method of estimating total microbial numbers (e.g., total DNA; i.e., molecular techniques). In addition, the PLFA signature from a given sample can show us a distribution of various types of microorganisms (Gram-positive bacteria, Gram-negative bacteria, arbuscular mycorrhizal fungi, saprophytic fungi, actinobacteria), and PLFA ratios are often used to measure community-level responses to water treatment. However, PLFA has a lower degree of resolution than 16S rRNA sequence analyses, so it is preferable to use both techniques combined to quantify the total amount of microbial biomass as well as the composition of that biomass in terms of the effects of alternative wetting and drying on microbial communities.

9.3. Statistical and Ecological Analytical Approaches

Sophisticated statistical methods, which can deal with each of these three types of variability - time, space, composition - are necessary for interpreting complex, multivariate datasets yielded from AWD rhizosphere studies. To visualise microbial community composition and the relationships of that community with its environmental predictors (such as moisture, proportion of carbon to nitrogen to phosphorus [C:N:P], and rates of enzyme reaction) the two most commonly used tools are principal component analysis (PCA) and redundancy analysis (RDA) (Zhou *et al*., 2010) [24] (Kuramae *et al*., 2012) [25]. Specifically RDA allows for the partitioning of the variation in community composition as it relates to multiple environmental predictors thereby providing a means for evaluating the degree to which moisture, nutrient (stoichiometric) ratio and physicochemical properties of the environment are structuring AWD induced rhizosphere communities.

Structural equation modelling allows for the possibility of testing different theoretical causal pathways between multiple interacting variables and has helped enable different applications of SEM to soil microbial ecology, which has been used by researchers to differentiate direct impacts of environmental variables from indirect impacts that are mediated through changes in microbial community composition and/or activity due to enzymatic processes (Zhou *et al*., 2010) [24]. For example, in relation to agriculture, SEM can be used in future applications to validate and evaluate the theoretical conceptual frameworks outlined in this review of the effects of alternate wetting and drying (AWD) methods on yield via N mineralisation rates, P pulse availability, and/or Bradyrhizobium nodulation efficiency. The use of network analysis provides an additional approach to provide additional information regarding how microbial communities organise themselves, as well as how the configuration of the network (connectivity, modularity, and/or keystone species) responds to AWD conditions.

9.4. Methodological Limitations and Analytical Challenges

Even though there have been many improvements to the methodologies used to study the effects of AWDs on groundnut rhizosphere stoichiometry and microbial community composition, there are still many limitations to our understanding of how these changes take place. Most of the research conducted to date has relied on destructive sampling designs, which look at community composition and activity; however, due to the fact that they only record data at a single point in time, they do not capture community succession, which is very rapid and varies considerably from moisture cycle to moisture cycle. The development of true time-series sampling designs that have enough temporal resolution to measure both the rapid Birch-like phase (hours to days) and the slower community succession (days to weeks) would greatly enhance the mechanistic understanding of the relationship between AWDs and microbial communities, but would require a much larger resource investment.

Another important limitation of current studies is a scale mismatch between laboratory and field experiments. Experiments conducted in controlled pots or microcosms, where the exact moisture cycle has been imposed on homogenized soil, do not accurately represent the conditions in the field where there is considerable spatial heterogeneity, preferential flow paths, and complex root-soil interactions. On the other hand, experiments conducted in the field with natural variability in precipitation will likely confound any effects of AWDs with other uncontrolled environmental variables. Some hybrid techniques have been used, including using rhizotrons in the field, mesh bags to exclude particular organisms, and in situ molecular sampling; however, each of these techniques also presents a series of methodological limitations that must be considered carefully in the design of the experiments.

9.5. Future Research Directions and Technological Opportunities

Researching the effects of Alternating Wet/Dry on the stoichiometry of Groundnut production, as well as on the function and community composition of the Rhizosphere has resulted in common priority areas (as summarized in Table 6). The first priority is to create Arachis hypogaea species Rhizosphere databases that integrate multi-omic datasets related to the growth and/or management of A.hypogeae in different soils and environments. These databases will enable development and validation of predictive models for the effects of AWD on Rhizosphere function.

Secondly, using precision soil moisture sensing and real-time monitoring of microbial activity through biosensor technologies or gas exchange systems will enable adaptive management of AWD based on the functional state of the Rhizosphere rather than on a set level of moisture.

Third is the application of synthetic ecology approaches to developing new microbial consortia of Bradyhizobium PSMs, and other taxonomic groups identified through network analysis, which can enhance resilience in AWD-grown Groundnut production through increasing functional redundancy for the critical nutrients cycling.

Lastly, there is a need for the development and validation of integrated models of the physiological responses of plants, stoichiometric dynamics of the Rhizosphere, and the functions of microbial communities that occur under the variable moisture conditions resulting from AWD that are needed to translate the mechanistic understanding provided by research into practical management information that can support sustainable Groundnut production.

Table 6: Research gaps, methodological limitations, and future research priorities in AWD rhizosphere stoichiometry and microbial ecology.

Research Gap / Limitation	Description and Challenge	Recommended Future Approach
Temporal resolution of microbial responses	Most studies use single-time-point sampling; miss short-term (hours-days) dynamics of Birch effect and community succession	Time-series sampling with high-frequency molecular profiling; paired transcriptomics and metabolomics
Lack of genotype-specific data for groundnut	Most rhizosphere studies use model plants (wheat, maize, rice); A. hypogaea-specific data are scarce	Dedicated rhizosphere metagenomics studies across groundnut varieties and growth stages
Integration of C:N:P stoichiometry with community data	Stoichiometric measurements rarely combined with high-resolution microbial community profiling in the same experimental system	Multi-omics approaches coupling nutrient analysis with metagenomics, metatranscriptomics, and metabolomics
Microcosm vs. field representativeness	Controlled laboratory AWD simulations may not capture field-scale spatial heterogeneity, crop canopy effects, and natural inoculum diversity	Long-term field experiments in multiple agro-ecological zones with concurrent laboratory mechanistic studies
Distinguishing active vs. dormant microbial fraction	Total DNA-based studies overestimate active community; dormant cells contribute to observed diversity	RNA-based (16S rRNA) and stable isotope probing (SIP) to target metabolically active populations
Phosphorus fractionation under AWD	Limited data on how organic and inorganic P fractions respond dynamically to moisture fluctuations in groundnut systems	Sequential P fractionation (Hedley scheme) combined with enzyme activity assays and isotopic labelling
Long-term stoichiometric feedbacks	Short-term experiments dominate; cumulative effects of repeated AWD cycles on soil C:N:P balance poorly characterised	Multi-year crop rotation experiments with comprehensive nutrient budgeting and microbial community monitoring

10. Figures

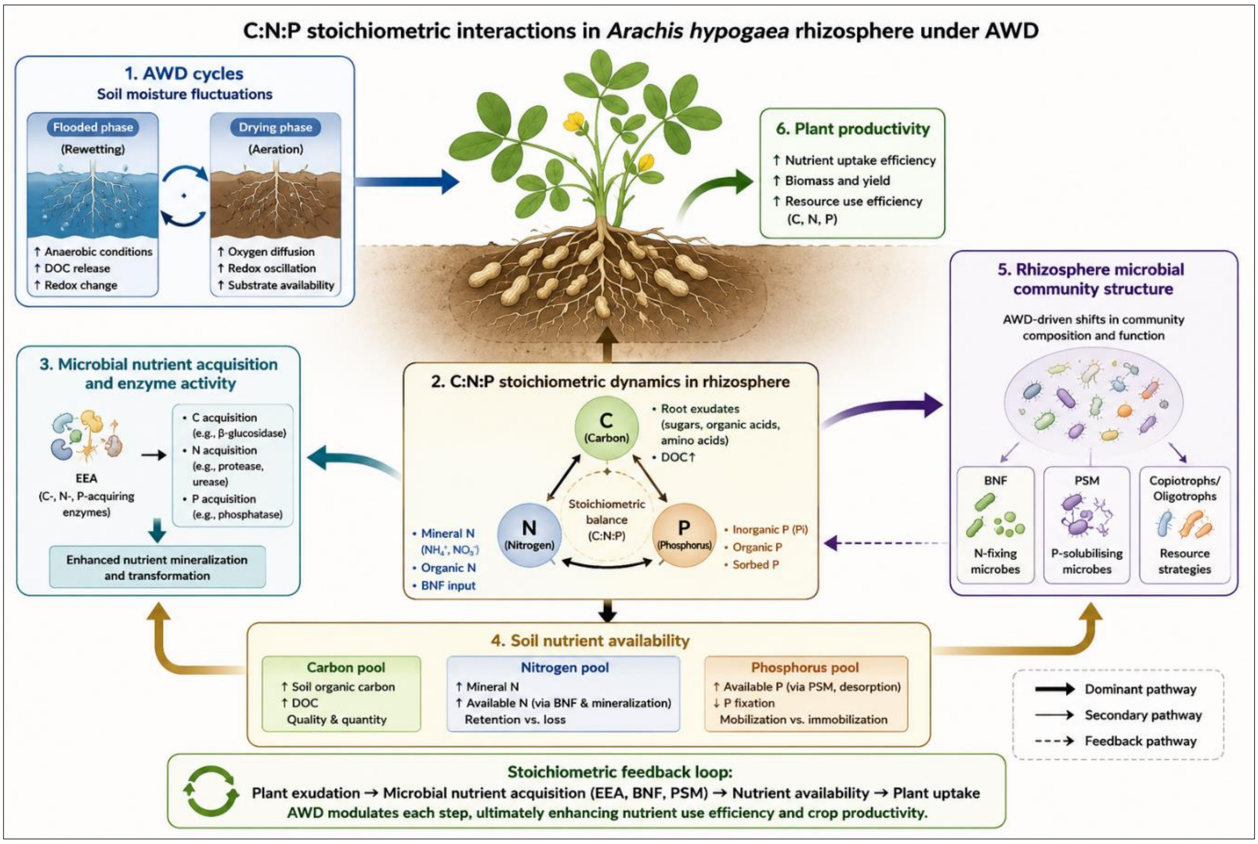

Fig 1: Conceptual diagram: C:N:P stoichiometric interactions in A. hypogaea rhizosphere under AWD

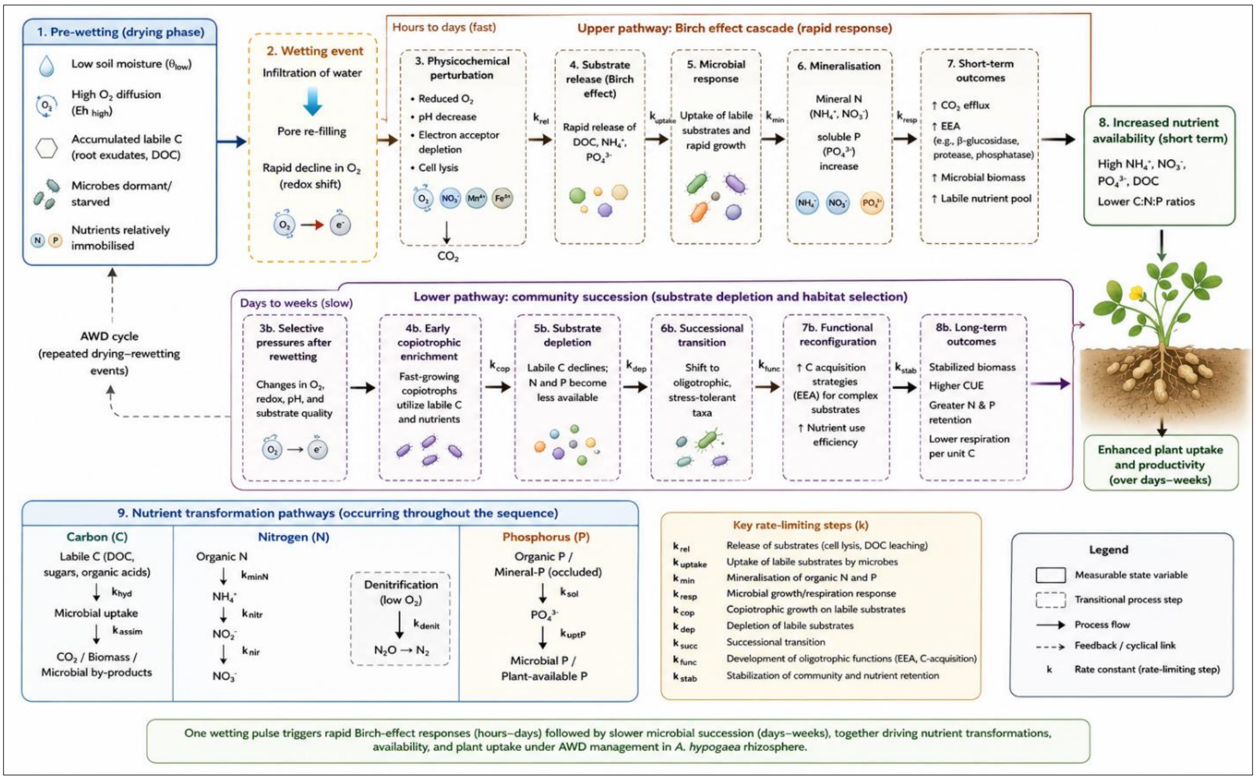

Fig 2: Flowchart: Effects of AWD on microbial community assembly and nutrient transformations

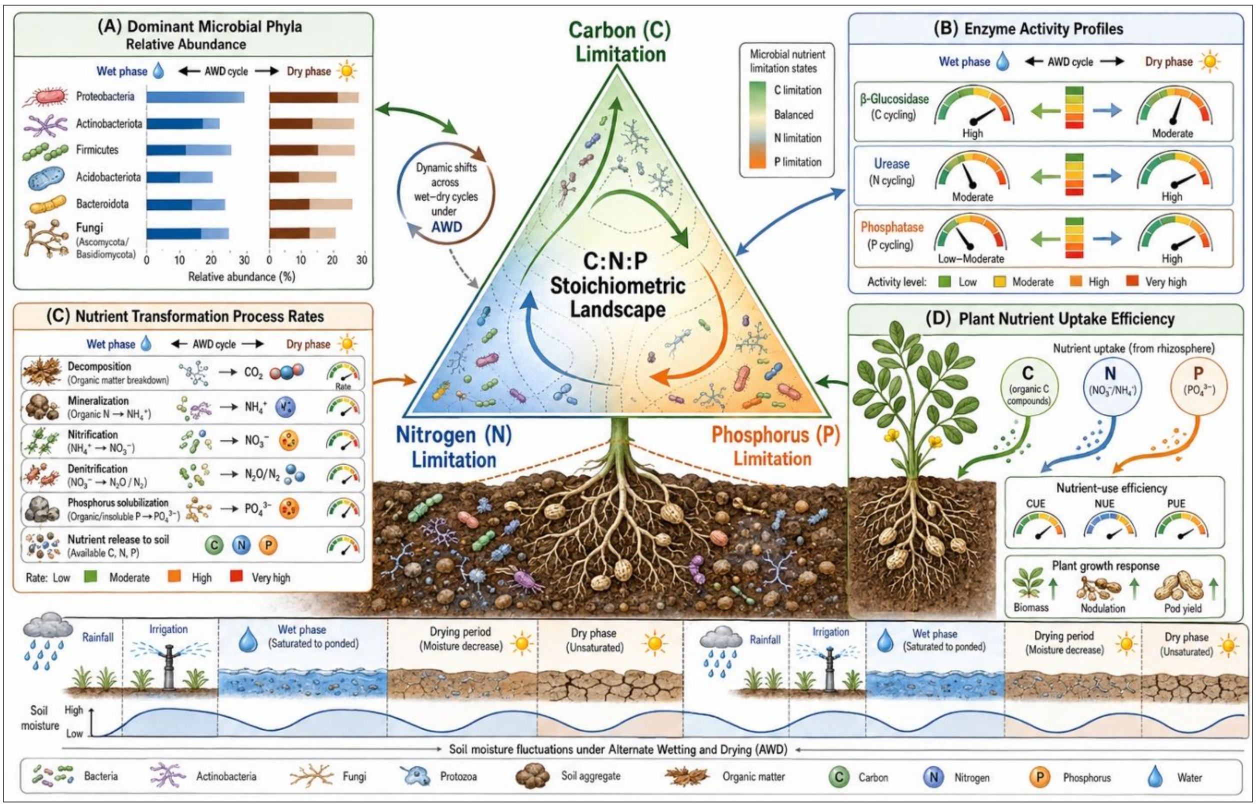

Fig 3: Mechanistic model: Interactions among rhizosphere microorganisms, nutrient cycling, and moisture fluctuations

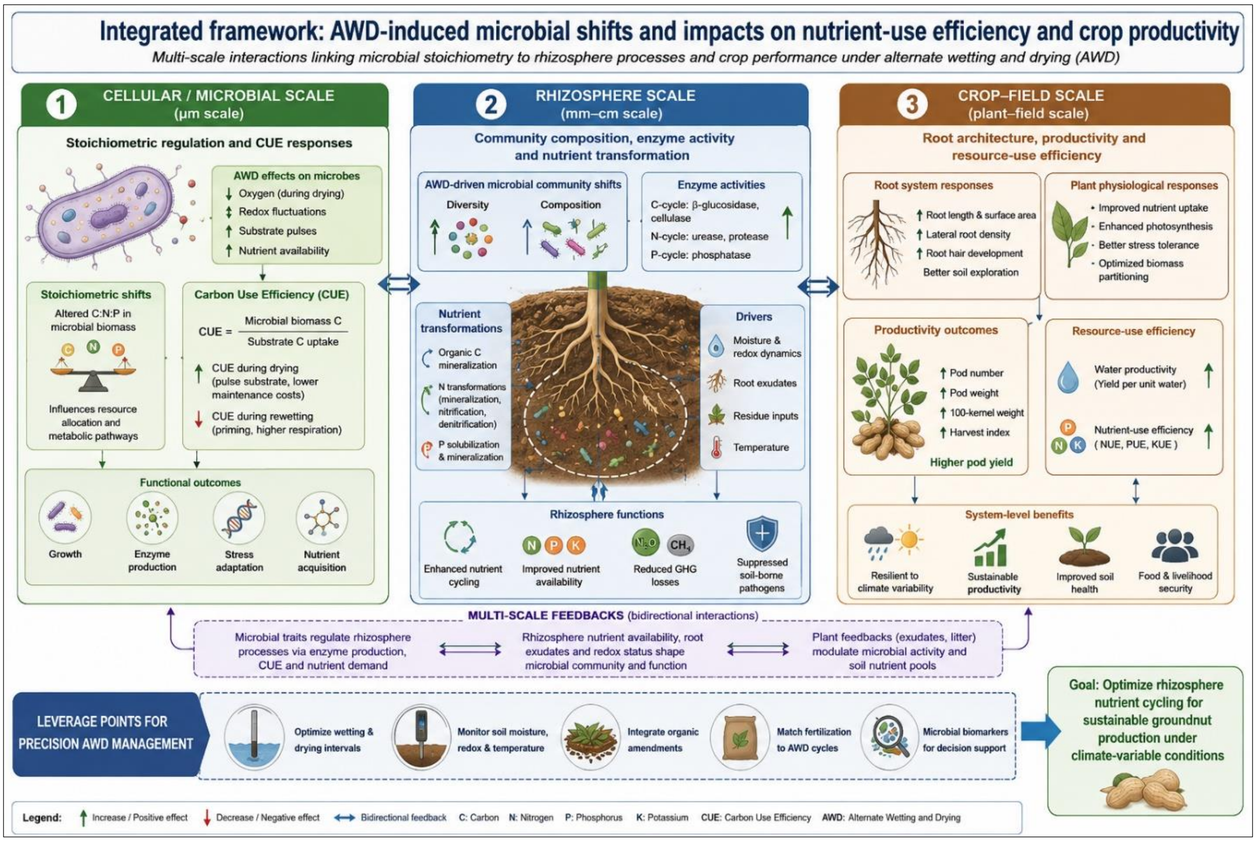

Fig 4: Integrated framework: AWD-induced microbial shifts and impacts on nutrient-use efficiency and crop productivity

11. Conclusion

The current state of the knowledge base about C:N:P stoichiometric relationships and their coupling to microbial community structure and function is now better understood in relation to the Arachis hypogaea root zone when exposed to alternating wet-dry periods through this review. The totality of evidence reviewed suggests that the AWD management system creates an environment where the stoichiometric balance of the rhizosphere is continually disturbed and restored, which has serious implications for both the ecological health of the rhizosphere's microbiome and the ability of the groundnut to produce food. Four main conclusions from this review can be summarised as follows: (1) AWD significantly modifies the stoichiometric configuration of groundnut rhizospheres via its effects on aggregation, redox state and microbial biomass turnover, establishing oscillating trends in mineralisation of nutrients – especially N & P. These trends represent opportunities and liabilities with respect to crop fertilisation depending upon the timing of the oscillations compared to the peak nutritional demand by groundnut. Second, the effect of alternating wetting and drying (AWD) on the microbial community in the rhizosphere of Arachis hypogaea exhibits a clear sequence of succession driven by the life history strategies of the microbial community. Immediately after rewetting, the community is dominated by copiotrophic Proteobacteria, while oligotrophic Actinobacteria become the dominant group during drying periods. This succession is reflected in the enzyme stoichiometry of the microbial community, providing diagnostic information about its nutrient limitation status.

Third, the stoichiometric coupling of the carbon, nitrogen, and phosphorus cycling pathways means that moisture-induced disturbances in one cycle can transmit to the other cycles in ways that cannot be fully assessed by examining any one cycle alone, highlighting the necessity of multi-nutrient, ecoenzymatic stoichiometry approaches for the characterization of the effects of AWD on rhizosphere functioning.

Fourth, the ability of AWD-managed groundnut rhizosphere ecosystems to deliver critical nutrient services—especially biological nitrogen fixation and phosphorus solubilization—depends greatly upon the functional redundancy of the community and the extent to which AWD management preserves the ecological niches of key specialist taxa such as Bradyrhizobium, arbuscular mycorrhizal fungi, and phosphorus solubilizing bacteria.

The above findings reveal that an effective agricultural management approach should utilize optimized AWD for maximising potential yield through a systems approach which considers the effect of soil physical properties, chemical properties and biological properties on the systems' performance. Therefore, avoiding severe drying during pod initiation and fill by either having adequate volume in moisture reservoir or frequent application of irrigation water in between irrigation events is critical to maintain nodular function (root nodules are what allow leguminous crops like groundnuts to draw nitrogen from the air and from the surrounding soil) and to maintain symbiotic relationships (mycorrhizae) between groundnut crops and beneficial fungi in the soil. Additionally, use of organic amendments (e.g. compost, vermicompost, manures, etc.) in conjunction with conventional fertilisers to improve both the WHC of the soil and the microbial habitat characteristics will mitigate against the intensity of stoichiometric disruption within each cycle of AWD, while at the same time reinforce the functional resilience of the rhizosphere microbiome. Accurate irrigation scheduling to synchronise the timing of irrigation events with the timing of peak crop nitrogen and phosphorus requirements, based on real-time measurements of soil moisture content as well as the added application of biofertilisers that are responsive to the addition of nitrogen and phosphorus to the soil, represent attainable means of maintaining or enhancing groundnut productivity under the reductions in water use made possible through the use of AWD.

The future of research will focus on collecting time-resolved, multi-omics datasets from large scale AWD studies conducted in area that represents groundnut growing conditions, and is also augmented with continuous data on moisture, nutrients and gas exchange at the site of study. A network-theoretic and SEM approach to these datasets will provide an opportunity to test causal hypotheses related to the pathways by which AWD affects crop nutrition through the rhizosphere microbiome. Process-based models that can simulate dynamic C:N:P stoichiometry in the rhizosphere of groundnut under changing moisture conditions will provide the necessary quantitative tools to take findings from specific studies and apply them more broadly to agro-ecological systems. Accomplishing these objectives will require a sustained effort in interdisciplinary collaboration across the disciplines of soil science, microbial ecology, plant physiology, agronomy and environmental science, but the results from evidence-based management practices that optimize groundnut production, soil health and efficient water use in an increasingly variable climate make this investment worthwhile.

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How to Cite This Article

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