

Quantifying the contribution of individual inputs used in Zero Budget Natural Farming

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RESEARCH ARTICLE

Quantifying the contribution of individual inputs used in Zero Budget Natural Farming

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Abstract

Zero Budget Natural Farming (ZBNF) in Andhra Pradesh promotes home-made, locally sourced, agrochemical-free inputs and regenerative land management techniques. Inputs consist of seed treatments (bijamrita), microbial inoculum applied either as a liquid foliar spray (liquid jiwamrita) or solid top dressing (solid jiwamrita) to the soil, and mulching (achhadana). However, some farmers do not use all the recommended inputs. There is a lack of evidence on the effects of partial adoption on the resulting yield and on the contributions of individual inputs to the performance of the overall approach. Controlled field experiments were established over two seasons across four agro-climatic zones. They consisted of five treatments. A *Standard ZBNF* treatment, which included application of all four ZBNF amendments (bijamirita, solid jiwamrita, liquid jiwamrita and dead mulch). The subsequent four treatments excluded one of the ZBNF inputs (*Minus Bijamrita*, *Minus Soilid Jiwamrita*, *Minus Liquid Jiwamrita*, and *Minus Dead Mulch*). Exclusion of each ZBNF input individually resulted in a significantly smaller yield than the treatment where all four inputs were used. However, exclusion of solid jiwamrita, liquid jiwamrita and mulching had a larger yield penalty than exclusion of bijamrita. Partial adoption could therefore impact the efficacy of the ZBNF system to deliver sustainable crop yields and satisfy food security. However, further research is needed to examine the effects of input exclusion in the long term, and possible interactions between different ZBNF inputs.

KEYWORDS

bijamrita, jiwamrita, natural farming, organic mulching, zero budget natural farming

1 | INTRODUCTION

Zero Budget Natural Farming (ZBNF) is a grassroots agrarian movement designed as a low-cost, farming

method that uses home-made, locally-sourced, amendments instead of relying on synthetic agrochemicals. The Indian government's National Mission on Natural Farming (Ministry of Agriculture & Farmers

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Welfare, 2024), states that natural farming currently covers 409,000 ha, across 8 states. The largest contribution is from Andhra Pradesh, SE India, and is currently being practised by c. 12% of farmers in the state. As well as being a locally and regionally based grassroots movement, ZBNF is also a state-government backed agricultural extension priority in Andhra Pradesh, more recently referred to as Andhra Pradesh Community Managed Natural Farming (APCNF). It is the intention that natural farming will be adopted by 6 million farmers across the state (Tripathi et al., 2018). Natural farming adoption is promoted by the not-for-profit organization Rythu Sadhikara Samstha (RySS). The aim of RySS is to create an “integrated institutional mechanism for all programmes, schemes and activities intended for farmer’s empowerment, encompassing welfare, development, capacity enhancement, credit flow, financial support, and allied empowerment activities” (RySS, 2020).

The amendments commonly used in ZBNF include *Bijamrita*, *Jiwamrita*, and *Achhadana*. Below we will outline the commonly held beliefs of ZBNF promoters and practitioners of these amendments, summarized in Figure 1. *Bijamrita* (a.k.a. *bijamrit*, *beejamarit*, *beejamrita*, *beejamruth*, *beejamrutha*) is a seed treatment applied either as a seed coating before sowing, or a root dip before transplanting. It is suggested that *bijamrita* can protect seeds, and roots of seedlings from soil borne diseases (Badiyala & Singh, 2021; Devarinti, 2016; Khadse & Rosset, 2019) and stimulate growth (Biswas, 2020; Gore & Sreenivasa, 2011). Common ingredients of *bijamrita* include: *Desi* cow dung and urine; CaCO_3 ; *Asafoetida*;

Phyllanthus emblica powder, ash and water (Badiyala & Singh, 2021; Biswas, 2020; Devarinti, 2016; Khangarot et al., 2022).

Jiwamrita (a.k.a. *jeewamrita*, *jeewamruth*, *jeewamrutha*, *jivamrita*, *jeevamrita*, *jeevamrit*, *jeevamruth*, *jeevamrutha*) is described as a fermented microbial inoculum. *Jiwamrita* can be in solid form (*Ghana jiwamrita*), usually applied as a top dressing, or in liquid form (*Dhrava jiwamrita*) as a top dressing or foliar spray. The premise of *jiwamrita* application is that all nutrients that a plant requires are present in the soil, just in unavailable forms. However, addition of beneficial microbes present in *desi* cow dung and undisturbed ‘virgin’ soil can liberate these nutrients, making them available for plants (Badiyala & Singh, 2021; Bishnoi & Bhati, 2017; Biswas, 2020; Gangadhar et al., 2020; Khadse & Rosset, 2019; Korav et al., 2020). Particular reference has been made to the application of N-fixing and P-solubilizing bacteria in *jiwamrita* (Boraiah et al., 2017; Devarinti, 2016; Gangadhar et al., 2020; Korav et al., 2020; Saharan et al., 2023; Santosha Gowda & Sudhir Kamath, 2021). It has also been suggested that application of *jiwamrita* will increase earthworm populations and activity, which will in turn increase nutrient availability (Badiyala & Singh, 2021; Bishnoi & Bhati, 2017; Khadse et al., 2018; Khangarot et al., 2022). Application of *jiwamrita*, particularly as a foliar spray, is also thought to suppress plant foliar diseases and pests (Ghosh, 2019; Khadse et al., 2018; Khangarot et al., 2022; Korav et al., 2020). Ingredients of *jiwamrita* can include: *desi* cow dung and urine; jaggery (unrefined cane sugar);

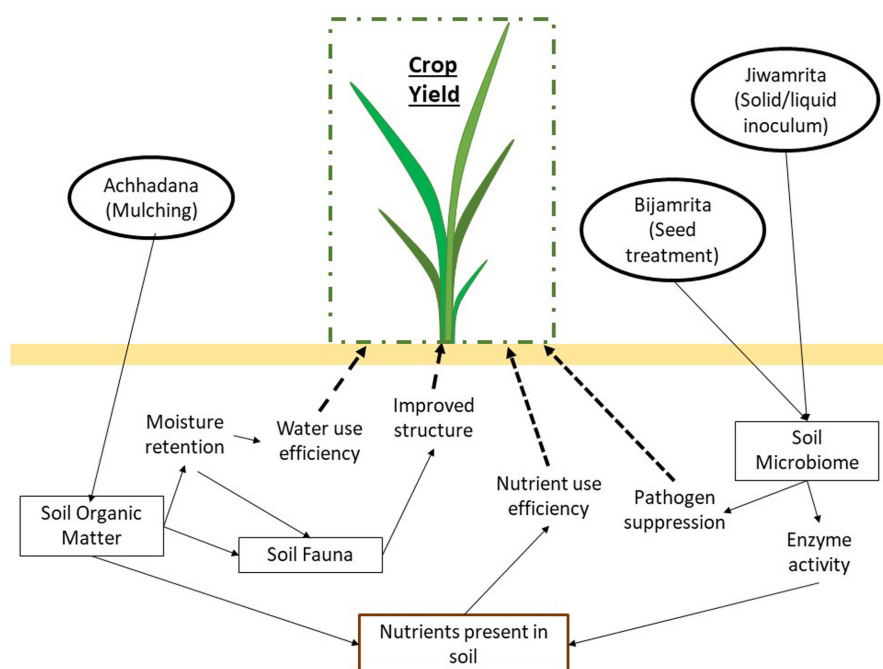


FIGURE 1 Perceived benefits of ZBNF inputs (in ovals) on crop yield.

gram (legume) flour; and topsoil from a native ‘virgin’ soil (uncontaminated, undisturbed soil from a forest for example) and, in the case of liquid jiwamrita, water is also added (Badiyala & Singh, 2021; Biswas, 2020; Khadse et al., 2018; Khadse & Rosset, 2019).

Achhadana (a.k.a. *acchadana*) refers to the practise of mulching, this is split into three components: (i) ‘*soil mulch*’ refers to the preservation of the topsoil by minimizing tillage; (ii) *live mulch* consists of intercrops and cover crops, including legumes; and (iii) *dead mulch* involves application of dry crop residues to the soil surface (Biswas, 2020; Khadse et al., 2018; Korav et al., 2020). Examples of dead mulch used in ZBNF include paddy straw and groundnut husks. Mulching is thought to improve the micro-climate of the soil, reduce evaporation, increase organic matter content, provide nutrients, protect topsoil, improve moisture retention, stimulate microbial activity, and suppresses weeds and pathogens (Badiyala & Singh, 2021; Bishnoi & Bhati, 2017; Biswas, 2020; Ghosh, 2019).

Controlled field experiments across Andhra Pradesh have revealed that the use of ZBNF inputs will not have an initial yield penalty compared with organic or conventional inputs (Duddigan et al., 2022, 2023). In addition, crop-cutting data with ZBNF and ‘non-ZBNF’ farmers ($n = 1531$) has reported a significant yield increase when using ZBNF practises and suggested that ZBNF can make a contribution to food security in the region (Bharucha et al., 2020). However, the ZBNF farms in these studies encompassed bijamrita, jiwamrita and achhadana amendments. This is important to note as some farmers adopt only a subset of these amendments (Bharucha et al., 2020). Partial adoption allows the farmers to experiment and thereby reduce the perceived potential risks of adopting ZBNF which is still a relatively new practise to them (Rose et al., 2021), and adopt ZBNF progressively to suit their local context. For example, ZBNF is often limited by the availability of mulch material and the *desi* cow breed (Khadse & Rosset, 2019) leading to partial adoption. In a study by Gupta et al. (2020), 23 per cent of ZBNF practitioners were found to be partial adopters.

There is a lack of evidence on the contribution of the individual ZBNF inputs to the whole ZBNF system (Korav et al., 2020). It is therefore unknown what effect partial adoption (exclusion of a particular input) will have on soil physico-chemical properties, and subsequent yield. There is therefore a need to design ‘evaluations that take into account diverging levels of adoption and types of adoption across different farms’ (Bharucha et al., 2020). To our knowledge, this is the first assessment of the contribution of each of the individual inputs in ZBNF.

Based on ZBNF promotional materials and perceived benefits of ZBNF, we formulated the following hypotheses to test during our research (Figure 1):

1. Exclusion of any single ZBNF input (bijamrita, jiwamrita, dead mulch) will lead to a reduction in yield, when compared with full adoption of standard ZBNF amendments
2. Jiwamrita (solid and liquid) stimulates microbial activity (enzyme activities) to mineralise nutrients and make them more available, improving yield
3. Dead mulch helps the soil to retain moisture which improves yield
4. Dead mulch, through provision of organic matter and moisture retention, stimulates earthworm populations that, in turn, improves the soil structure

2 | MATERIALS AND METHODS

2.1 | Study area

Andhra Pradesh lies on the Southeastern coast of India. More details of the study area can be found in Duddigan et al., 2022, 2023. Five individual controlled field experiments were established across the state in four of the six agro-climatic zones of Andhra Pradesh (Figure 2a). These include sites in the cooler district of *Visakhapatnam* in the North Coastal Zone; *Krishna* and *Prakasam* districts in the lowland valley of the Krishna Zone, *Nellore* district in the Southern Coastal Zone, and *Anantapur* district in the warmer Scarce Rainfall Zone. Details of the soil texture, crop selections, and *rabi* meteorological data at each site can be found in the supporting information (Table S1.)

2.2 | Experimental design

The experiment was co-designed with expert local knowledge (see Duddigan et al. (2022) for details on the co-design process). ZBNF has already been compared with conventional and organic alternatives (Duddigan et al. 2022, 2023) and therefore the focus of this experiment was to assess the impact on ZBNF yield if one of the inputs were not used, which is common in partial adoption. With this aim in mind, the co-designed experiment comprised five treatments (see below). In this instance, the standard ZBNF treatment is acting as the control in this experimental design.

- (i) Standard ZBNF, which included application of all four ZBNF amendments (bijamrita, solid jiwamrita, liquid jiwamrita and dead mulch).

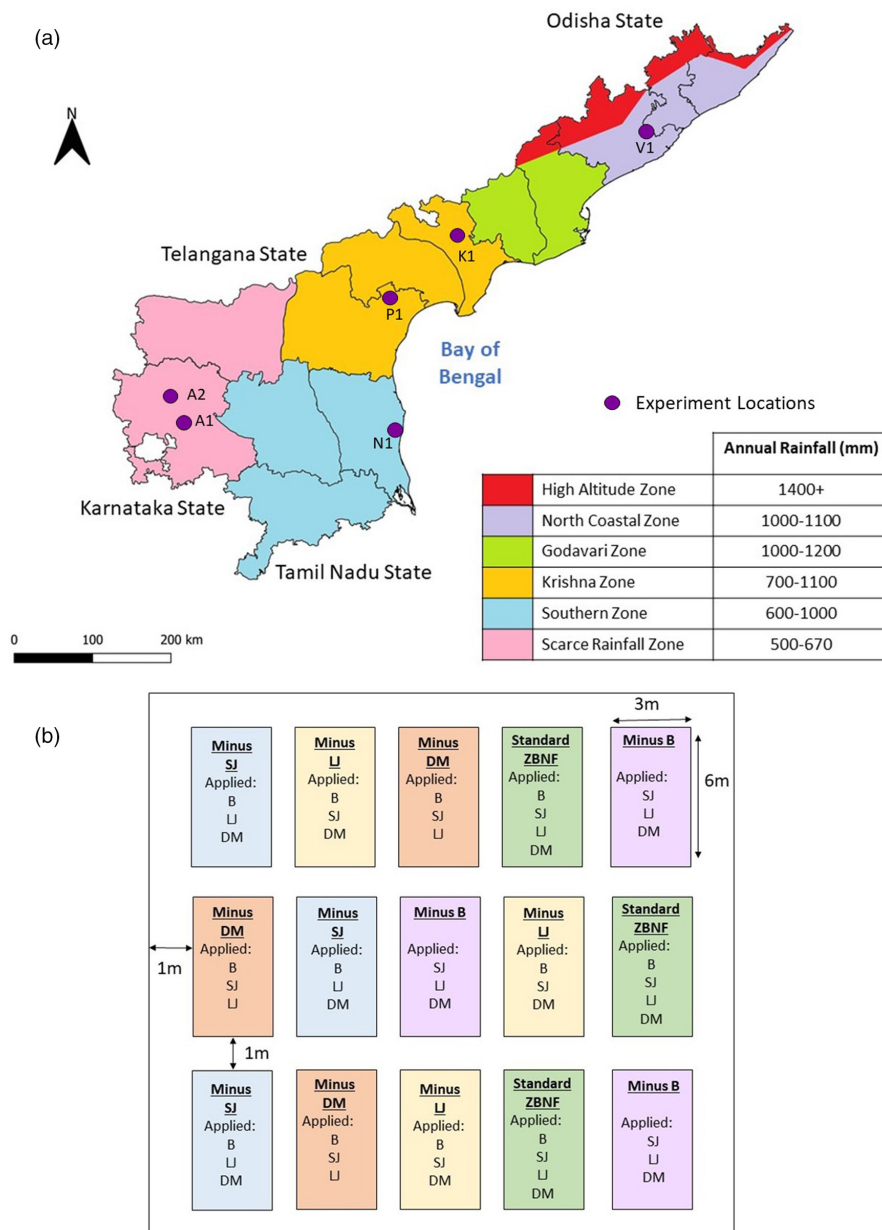


FIGURE 2 (a) Locations of field experiments in Andhra Pradesh with agro-climatic zones, adapted from Reddy et al. (2018); (b) Example experimental design. B, biamrita; DM, dead mulch; SJ, solid jiwamrita; LJ, liquid jiwamrita.

- (ii) Minus biamrita, which excluded the use of biamrita (but applied the other three amendments).
- (iii) Minus solid jiwamrita, which excluded the use of solid jiwamrita (but applied the other three amendments).
- (iv) Minus liquid jiwamrita, which excluded the use of liquid jiwamrita (but applied the other three amendments).
- (v) Minus dead mulch, which excluded the use of dead mulch (but applied the other three amendments).

Each treatment was applied to three replicate 6 m × 3 m plots (Figure 2b) in a randomized block design (15 plots in total per experiment). Each of the five experiments had a unique randomized block design. Experiments were continued on the same sites across two seasons, *kharif*

(monsoon) and *rabi* (winter) of 2019–2020, except for Anantapur where the experiment had to be relocated because of logistical constraints (See Table S1). As a result, one Anantapur experiment was conducted in *kharif* only, and the other for *rabi* only.

Liquid jiwamrita contains a large volume of water so the equivalent volume of water was applied to the Minus liquid jiwamrita treatment to ensure that treatment effects were because of the exclusion of the amendment rather than simply the exclusion of direct water application.

Crop selection was based on local practice in the surrounding areas of the farm in which the experiment was being conducted, and therefore varied across districts and seasons (Table S1). Crops were hand sown or transplanted. Plant spacing and quantities of amendments applied

varied depending on the main crop in question, according to protocols issued by RySS, to be in line with current practise (Table S2). As per ZBNF practise, alternate rows of intercrops were also sown (Table S1) but amendment applications were made according to the main crop.

2.3 | Yield

Yield measurements were made on the main crops only, not the intercrop. Yield was considered as the mass of produce obtained from each plot, as it would be taken to market, rather than whole plant biomass (see Duddigan et al. 2022). To make yield data comparable between crop types, yield data was z transformed (Equation 1).

$$z = \frac{x_i - \bar{x}}{S} \quad (1)$$

Equation 1 Z score transformation.

Where z is normalized yield for a single plot, x_i is the plot yield for the single plot, \bar{x} is the mean yield of all 15 plots on the given farm experiment, and S is the standard deviation of the yield of all 15 plots on the given farm experiment. If the yield of a single plot is equal to the mean yield of all 15 plots on a given experiment, then $z=0$. If the plot yield is below the mean yield of all 15 plots, then $z<0$. Finally, if the plot yield is above the mean yield of all 15 plots, then $z>0$.

2.4 | Soil sampling and analysis

Soils were sampled three times in each of the two seasons: an Initial sample taken before any amendments were applied in that season; a Mid-Season sample taken halfway through the growing season of the main crop; and a Post-Harvest sample taken after all product and biomass has been harvested from the plots.

Five soil samples were taken from the central 5 m × 2 m (to avoid boundary effects) in each plot in a 'W' formation. These were then homogenized to form one composite sample per plot for each point in the season. During the *rabi* season, a subsample was also frozen for next generation sequencing (NGS) (see below). The remaining sample was sent off to be analysed for pH, organic C, electrical conductivity, extractable nutrients (N, P₂O₅, K₂O, Cu, Fe, Mn and Zn) and enzyme activity (Dehydrogenase, urease, acid and alkaline phosphatase). All nutrient and enzyme analyses were conducted according to Ramana Reddy et al. (2012) by the Regional Agricultural Research Station at Acharya N.G. Ranga Agricultural University (Tirupati, Andhra Pradesh). Details of the methods can be found in the Table S3.

2.5 | In field measurements

In addition to soil sampling, observations were also made in the field at the initial, mid-season and post-harvest points of each season. These included: soil moisture; soil temperature; infiltration rate; bulk density; earthworm abundance and plant biometrics. The methods used to measure these can be found in Duddigan et al. (2023).

2.6 | Root simulators

Plant root simulator (PRS[®]) probes (Western Ag Innovations, Saskatoon, Canada) estimate supply rates of nutrients to plant roots at a soil depth of approximately 3–9 cm. The probes consist of ion exchange resin membranes held in plastic supports (Western Ag, 2022). Anion probes have a positively charged membrane to simultaneously attract and adsorb all negatively charged anions (e.g. NO₃⁻, H₂PO₄⁻, HPO₄²⁻, SO₄²⁻, micronutrients etc.). Cation probes have a negatively charged membrane to attract and adsorb all cations (e.g. NH₄⁺, K⁺, Ca²⁺, Mg²⁺ etc.). Four cation and four anion probes were placed, in pairs, in each plot in randomly selected locations within the central 5 m × 2 m. They were put in place 2 weeks before the mid-season sampling and removed 2 weeks after the mid-season sampling. The four anion probes and four cation probes were then bulked together for analysis to give a single value for each ion per plot. Results were reported in micrograms of ion per 10 cm² (the size of the membrane) during the time the probes were in place. This cannot be subdivided into shorter time periods (e.g. per day) because it cannot be assumed that the supply rate to the membrane is linear over time.

2.7 | NGS and bioinformatic analysis

NGS of 16S rDNA, ITS1 and ITS2 amplicons of soil extracted DNA and subsequent bioinformatic analysis was conducted by Genotypic Technology (Bangalore, India) as described in the Supplementary Information or on the Genotypic Technology website (Genotypic Technology, 2023).

2.8 | Statistics

For variables where data was collected more than once in a season (initial, mid-season and post-harvest), a repeated measures analysis of variance (ANOVA) with least significant difference (LSD) post hoc testing was performed for each season of data (kharif and rabi) in Genstat

(Version 19.1). Treatment was used as a factor (standard ZBNF, minus bijamrita, minus solid jiwamrita, minus liquid jiwamrita, minus dead mulch) farm/plot as a block structure, and point in season (initial, mid-season, post-harvest) as time points. District was not included as there was only one farm per district in each season. The seasons were kept separate because the experiments changed their main crop, and therefore input quantities, each season.

For yield, PRS[®] probe anions/cations, and plant biometric data, a repeated measures ANOVA was not used as there were no repeats in a season; they were only measured once. Therefore, a restricted maximum likelihood (REML), mixed effects model, with interactions, and Tukeys post-hoc testing was used to determine the effect of treatments on yield and plant biometrics (Table 1) in Minitab (Version 20). District, treatment, and crop selected (legume or non-legume) were classified as fixed factors, and farm as a random factor. Two way interactions were also included in the REML mixed effects model. However, three way interactions between treatment x season x crop were not possible because of the lack of replicates in each combination. For example, some regions only had legumes as their main crop and others only had non-legumes (see supplementary information).

The influence of treatments on the soil microbial community structure was examined using non-metric multi-dimensional scaling (nMDS) and analysis of similarity (ANOSIM), based on a Bray Curtis similarity matrix using Primer (Version 6). Firstly, all farms were included in one analysis to look at district (farm) differences, then each

farm was analysed separately to examine any differences between treatments and point in the season (initial, mid-season, post-harvest). An nMDS based on a Euclidean distance similarity matrix was also conducted for the soil physico-chemical data obtained at the same corresponding time points (bulk density, soil temperature, soil moisture, infiltration rate, pH, electrical conductivity, organic C, extractable N, P₂O₅, K₂O, Cu, Mn, Fe and Zn).

A BIOENV analysis, using Spearman's rho to quantify the association between two resemblance matrices was conducted in Primer (Version 6). In our case, this was a Euclidean distance matrix of soil physico-chemical properties and a Bray Curtis similarity matrix of microbial community structure (16s, ITS1 or ITS2), described above. A global BEST test was then conducted to identify the subset of soil physico-chemical properties that have the highest rho value, and the significance of the relationship of this subset with the microbial community similarity matrix in question (16S, ITS1 or ITS2).

3 | RESULTS AND DISCUSSION

3.1 | Effect of input exclusion on yield

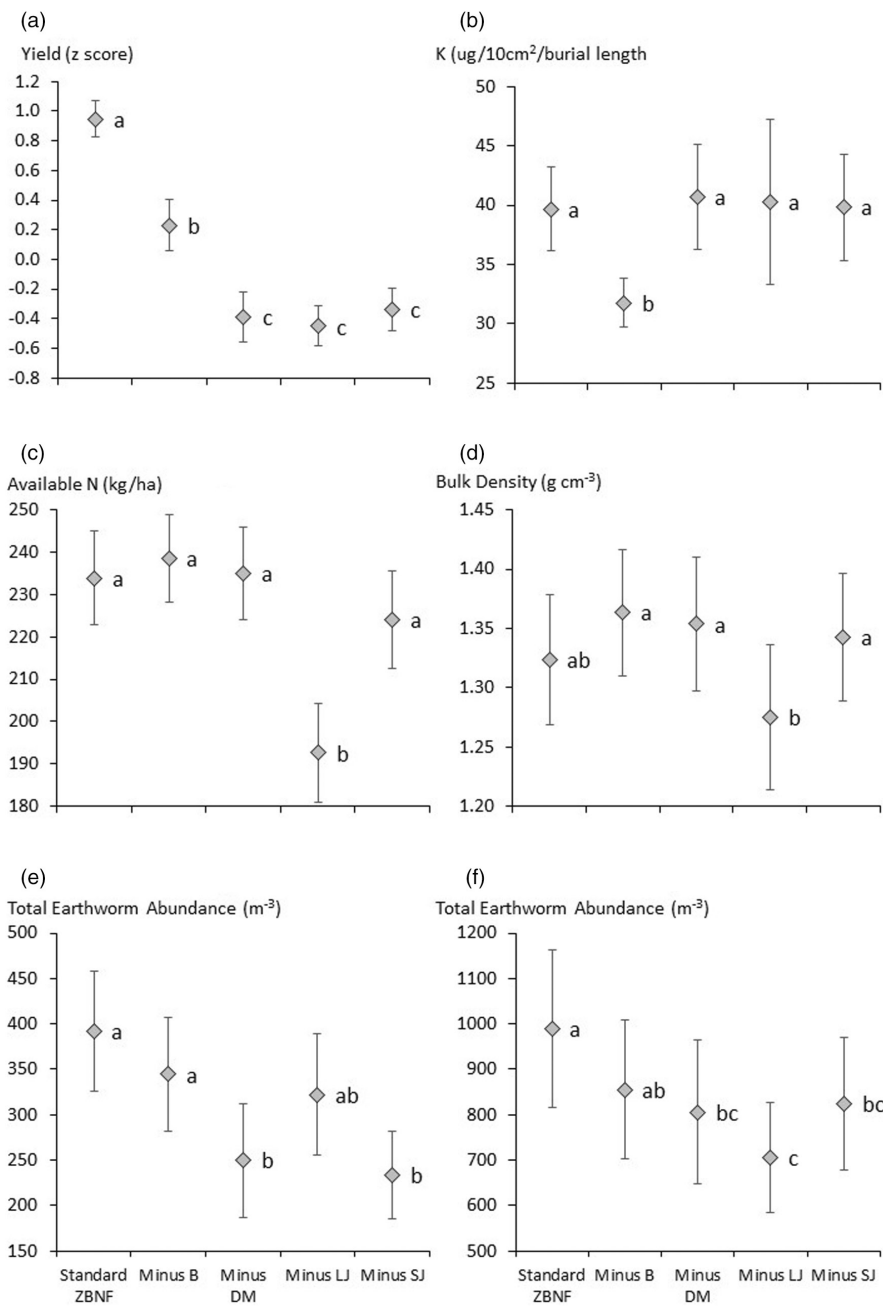
The first hypothesis we tested was that exclusion of any single ZBNF input (bijamrita, jiwamrita, dead mulch) will lead to a reduction in yield, when compared with full adoption of standard ZBNF amendments. Our results showed that exclusion of any of the four ZBNF

| Factor | Type | Number of levels | Levels |
|----------------------|-----------------------------|------------------|---|
| Farm | Random (nested in district) | 6 | A1; A2; K1; N1; P1; V1 (See Figure 2a for locations) |
| District | Fixed | 5 | Anantapur; Krishna; Nellore; Prakasam; Visakhapatnam |
| Treatment | Fixed | 5 | Standard ZBNF; Minus Bijamrita; Minus Dead Mulch; Minus Liquid Jiwamrita; Minus Solid Jiwamrita |
| Crop (legume or non) | Fixed | 2 | Legume; Non-Legume |
| Season* | Fixed | 2 | Kharif; Rabi |
| Treatment × district | Interaction | | |
| Treatment × crop | Interaction | | |
| Treatment × season* | Interaction | | |

TABLE 1 Summary of yield REML mixed effects model with treatment, district, season and crop variety as factors.

*Season was not included as a factor in PRS[®] probe data as they were only used in the rabi season.

FIGURE 3 Effect of ZBNF input exclusion (a) yield (z transformed) over two seasons; (b) PRS© probe K in rabi season; (c) available N in rabi season; (d) bulk density in kharif season; (e) total earthworm abundance in kharif; and (f) total earthworm abundance in rabi season. Error bars represent standard error. Treatments that are labelled with the same lower-case letter are not significantly different ($p > .05$) according to REML mixed effects model and Tukey's post-hoc testing (yield and PRS© K) or according to repeated measures ANOVA and least significant difference post-hoc testing (N, bulk density, earthworm abundance). Only variables with a significant treatment effect are shown (see Table S5 for analysis of all variables) Minus B, bijamrita excluded, Minus DM, dead mulch excluded, Minus LJ, liquid jiwamrita excluded, Minus SJ, solid jiwamrita excluded.



inputs resulted in a significantly ($p < .05$) smaller yield than the standard ZBNF treatment (Figure 3a). Partial adoption could therefore impact on the success of the ZBNF system to deliver sustainable crop yields and satisfy food security. RySS aims to give farmers scope to experiment with the methods and adopt ZBNF progressively. For example, farmers can begin by using ZBNF as a form of input substitution (Bharucha et al., 2020). Almost one quarter of ZBNF practitioners are thought to be partial adopters (Gupta et al., 2020), omitting at least one ZBNF input. Interviews with non-ZBNF farmers revealed that many are reluctant to adopt ZBNF practises for fear of poor yield or overall crop failure (Kumar et al., 2020).

Therefore, partial adoption is sometimes preferred by new ZBNF adopters to reduce the risks with converting to ZBNF practises from conventional or organic farming (Rose et al., 2021). This decision, however, could have repercussions, because making the decision to omit a single ZBNF input (bijamrita, solid jiwamrita, liquid jiwamrita, dead mulch) may be to the detriment of crop yield compared with the standard ZBNF treatment that encompasses all four inputs.

It has been proposed that the current number of cattle in India cannot support the required manure application if ZBNF was to be adopted at scale (Korav et al., 2020). Therefore, if partial adoption is a result of limited resources, such as *desi* cow manure, then our results suggest

that there will be a smaller yield penalty if bijamrita were excluded from the ZBNF system than solid or liquid jiwamrita (Figure 3a). So recommendations can be made concerning which amendments could be prioritized in partial adoption.

There were no significant interactions between treatment and district, or crop type (legume vs. non-legume), suggesting that the treatment effects we have observed are insensitive to local context (Table S4) and exclusion of any particular input in a partial adoption ZBNF system has similar effects regardless of crop type or location.

3.2 | Exclusion of bijamrita

It is commonly implied that bijamrita contains 'beneficial microorganisms', along with cow dung and cow urine, which protects crops from soil-borne pathogens (Bishnoi and Bhati, 2017; Khadse et al., 2018; Korav et al., 2020). However, exclusion of bijamrita in our experiments had no significant effect on the soil microbial community structure (bacterial or fungal, Table 2), diversity (Table S7) or enzyme activity (Table S5). It is important to note that it was largely above-ground insect pests that were damaging crops in our experiments, rather than soil-borne diseases (Table S9). Therefore, testing the efficacy of bijamrita on disease suppression is difficult in the farms where we conducted our experiments. Further investigation is needed on the impact of bijamrita on pest and disease suppression is needed in order to fully assess its efficacy.

It has been suggested that bijamrita aids seed germination (Sharma et al., 2020), and stimulates plant growth (Biswas, 2020). This is in concordance with our results that exclusion of bijamrita resulted in significantly smaller yields than the standard ZBNF treatment. However, the minus bijamrita treatment had a significantly greater yield than the treatments that excluded dead mulch, solid jiwamrita, or liquid jiwamrita (Figure 3a). This result suggests that bijamrita is the least important ZBNF input when it comes to yield outcomes during the first year of ZBNF adoption.

The only significant treatment effect observed in the PRS[®] probe data from the rabi season was on K (Table S6).

Significantly less K was found in the rabi season on the plots where the bijamrita was excluded (Figure 3b). The bijamrita used in ZBNF can contain ash (Ghosh, 2019; Keerthi et al., 2018). This ash input could account for the K content reported by the PRS[®] probes being smaller in the minus bijamrita treatment compared with the other treatments. Therefore, nutrient provision resulting from bijamrita input may be a result of direct application, rather than a result of enhanced microbial activity. This finding also means that, although exclusion of bijamrita has the lowest yield penalty, compared with standard ZBNF in our study, bijamrita may have a more important influence in K limited systems or on crops with a high K requirement.

3.3 | Exclusion of jiwamrita (solid and liquid)

Promoters and practitioners of ZBNF often claim that all nutrients needed by crops are present in the soil, and that the application of beneficial microorganisms present in jiwamrita catalyses the release of nutrients which would otherwise be unavailable to plants (Biswas, 2020; Korav et al., 2020). This led to our hypothesis that Jjiwamrita (solid and liquid) stimulates microbial activity through enzyme activities to mineralise nutrients and make them more available, thus improving yield. However, our research shows that, while exclusion of solid or liquid jiwamrita resulted in a crop yield penalty (Figure 3a), omission of these inputs had no significant effect on the soil microbial community structure (bacterial or fungal, Table 2), microbial diversity (Table S7) or enzyme activity (Table S5) compared with the standard ZBNF treatment.

Plant-growth promoting microorganisms can act as: (i) biofertilizers providing nutrients for the plant via symbiotic or associative pathways, (ii) phytosimulators producing plant hormones promoting plant growth directly; or (iii) biological control agents, protecting plants from phytopathogens (Trabelsi & Mhamdi, 2013). Enhanced yield by microbial inoculants has been linked in some cases to enhanced nutrient uptake and improved nutrient status of the plant (Calvo et al., 2014). A yield increase has been observed in *Capsicum annum* with

TABLE 2 Effect of amendment exclusion on soil microbial community. Analysis of similarity (ANOSIM) *p*-values of pairwise comparisons of standard ZBNF treatment vs. each exclusion treatment.

| | | Minus Bijamrita | Minus liquid Jiwamrita | Minus solid Jiwamrita | Minus dead mulch |
|---------------|------|-----------------|------------------------|-----------------------|------------------|
| Standard ZBNF | 16s | 0.621 | 0.344 | 0.416 | 0.536 |
| | ITS1 | 0.377 | 0.915 | 0.079 | 0.187 |
| | ITS2 | 0.450 | 0.751 | 0.858 | 0.026 |

jiwamrita application, compared with yield without jiwamrita application (Boraiah et al., 2017; Gangadhar et al., 2020). It has been suggested that this was a result of increased abundance of N-fixers and P-solubilisers in jiwamrita treated soils (Boraiah et al., 2017; Gangadhar et al., 2020).

N fixing bacteria include those found in genus *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Serratia* and *Streptomyces* (free living) and also the genera that comprise the rhizobial symbionts. P solubilisers include species in the genus *Bacillus*, *Pseudomonas*, *Rhizobium*, *Aspergillus* and *Penicillium* (Patel & Panchal, 2020). There was no significant treatment effect on the relative proportion of any genus we have listed above (Table S10) suggesting that N-fixing or P solubilisers are not differentially promoted by the ZBNF inputs. However, this is not an exhaustive list, nor can we assume that every species in each genus perform these functions consistently. Further research into the function of the species found in ZBNF systems, or direct measurement of N-fixation or P solubilization is needed to assess this further. Exclusion of solid and liquid jiwamrita also had no significant effect on the number of root nodules on groundnut plants, compared with the standard ZBNF treatment (Table S11).

The heterogenous nature of the soil environment makes it difficult for introduced microbial inoculants to establish a niche for survival and compete with the soil community that occupies the agricultural soil they are applied to (Frew, 2021; Khare & Arora, 2015; Lugtenberg & Kamilova, 2009). There was no significant difference between treatments in dehydrogenase activity (Table S5). Dehydrogenase is closely related to microbial biomass (Wolinska & Stepniewski, 2012), which suggests that any microbes applied to the soil in jiwamrita did not survive. This may account for the lack of a significant difference in microbial community structure between the standard ZBNF and the minus solid and liquid jiwamrita treatments (Table 2). There was a significant difference in the communities observed in each of the five experiments (Figure S2). These differences were related to differences in soil moisture, bulk density and extractable K_2O , Mn and Fe according to BIOENV and BEST analysis (Table S12). This may account for there being no treatment effect on microbial community structure, as the soil properties associated with microbial community structure at the regional level (e.g. Mn and Fe) were not influenced sufficiently by the treatments to be influential at the scale of the local experiment.

It has been suggested that ZBNF systems are likely to be more deficient in N than conventional systems (Smith et al., 2020). However, the application of liquid jiwamrita has been reported to improve yield through provision of

macronutrients, including N (Swami et al., 2021). In addition, Duddigan et al. (2023) found that there was no significant difference in extractable N contents in the soil of ZBNF, compared with conventional treatments in experiments that were conducted on the same land over three seasons. In this study, exclusion of liquid jiwamrita resulted in significantly lower concentrations of available N in the rabi season compared with all other treatments (Figure 3c; Table S5). This finding indicates that liquid jiwamrita is an important contributor of N to soils under ZBNF practice. However, it is likely that this contribution is a result of direct application of N in the urine included in liquid jiwamrita, rather than the liquid jiwamrita acting as a bio-stimulant. This reduction in available N in the minus liquid jiwamrita treatment was not enough to result in a significantly smaller yield compared with the minus solid jiwamrita, minus bijamrita and minus dead mulch treatments (Figure 3a,c) in the short term. In addition there was no significant interactions between yield and whether the main crop was a legume or not. Further investigation over longer periods of time is needed to fully understand the provision of N in ZBNF systems.

It has been suggested that ZBNF practises reduce the soil bulk density (Smith et al., 2021). Exclusion of liquid jiwamrita, however, resulted in a significantly lower bulk density than the other exclusion treatments (Figure 3d; Table S5). This could be a result of the application method of this amendment. Liquid jiwamrita is applied at regular intervals, often as a foliar spray, by hand spraying. Therefore application involves the practitioner walking over the soil regularly which could compact the soil and reduce the bulk density. Although an equivalent amount of water was applied to the minus liquid jiwamrita treatment, this was generally applied by a practitioner stood at the side of the plot (i.e. with a watering can) so the soil was not necessarily walked over in the same manner.

3.4 | Exclusion of dead mulch

We hypothesised that dead mulch helps the soil to retain moisture, thus improving yield. Organic mulching with crop residues, such as straw, has been found to suppress weeds and regulate soil temperature and moisture to improve crop yield (Chavan et al., 2009; Chen et al., 2007; Kader et al., 2017; Korav et al., 2020). In our research, however, despite a significant yield penalty from excluding dead mulch from the standard ZBNF practice (Figure 3a), there was no significant treatment effect on soil moisture or temperature (Table S5). It was observed by Duddigan et al. (2023) that ZBNF practises increase soil moisture, and subsequently decrease soil temperature, compared with conventional and organic

systems. Our findings here suggest that this is a result of the combination of ZBNF inputs. Exclusion of just one of the ZBNF inputs dead mulch (organic residue), bi-jamrita (which contains dung), solid jiwamrita (which contains dung) and liquid jiwamrita (which contains water) is not enough to significantly reduce the soil moisture content compared with the standard ZBNF treatment (Table S5). This is an unexpected result, particularly as mulching is usually found to adjust albedo and reduce evaporation in arid regions (Liu et al., 2014; Tuure et al., 2021), which can influence soil temperature and moisture.

We hypothesised, and it has been suggested in the literature, that mulching increases soil carbon content, subsequently improving soil physical condition, which in turn stimulates growth of earthworms and beneficial microbes (Kumar et al., 2020). Exclusion of dead mulch resulted in a significantly different ITS2 community compared with the standard ZBNF treatment (Table 2). Despite there being no significant treatment effect on soil organic carbon, exclusion of dead mulch resulted in a significantly lower earthworm abundance than the standard ZBNF in both the kharif (Figure 3e) and rabi (Figure 3f) seasons. However, this decrease in earthworm abundance does not appear to have had a significant effect on the soil bulk density (Figure 3d). In addition, exclusion of the solid and liquid jiwamrita also resulted in a significant decrease in earthworm abundance. This is in concordance with Veeresh and Narayana (2013) who observed that applications of cow dung and jiwamrita increased earthworm abundance during treatment of agro-industrial waste.

3.5 | Synergies between inputs

It is uncertain if it is the particular combination of ZBNF inputs, and synergies between them, that supports the efficacy of ZBNF or if the effects are additive. Some suggested synergies between ZBNF inputs have been put forward in the literature. For example, it has been suggested that application of liquid jiwamrita can enhance nutrient release from farmyard manure application through increased microbial activity (Manjunatha et al., 2009). It has also been proposed that jiwamrita can stimulate decomposition and nutrient release from mulch (Gangadhar et al., 2020). For example, depending on the C:N ratio, decomposition of straw is stimulated by N supply which could be provided by urea in the jiwamrita (Wang et al., 2021). In order to explore these interactions, a different experimental design which includes treatments of each input being used alone, alongside the standard ZBNF treatment and an unamended control treatment will allow examination of whether the standard ZBNF yield is greater than the sum

of the parts. In addition, long term studies would also be beneficial when examining the longevity of partial adoption of ZBNF amendments. For example, if the soils become mined of a particular nutrient then the importance of a specific amendment which provides that nutrient may increase with time.

4 | CONCLUSIONS

Here we have shown that exclusion of a single ZBNF input can lead to a significantly smaller yield. This finding has implications for farmers who are partial adopters of ZBNF during the early stages of transition. The exact mechanisms accounting for the contributions of each of the amendments are still unclear. However, our initial research suggests that beneficial microorganisms in the amendments are unlikely to influence crop yield in the short term, despite this mechanism often being highlighted by ZBNF promoters. Further research is needed to examine the long term effects of the contribution of each of the ZBNF inputs to the system as a whole, and possible interactions between these inputs.

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CONFLICT OF INTEREST STATEMENT

Rythu Sadhikara Samstha (RySS), the affiliation of authors ZH, KJ, HK, RS, RS, VT and HPV, are responsible for promotion of ZBNF across Andhra Pradesh.

DATA AVAILABILITY STATEMENT

Available here: [10.5281/zenodo.10853526](https://doi.org/10.5281/zenodo.10853526).

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