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Original article

Short-term impact of an occasional tillage on microbial communities in a Vertosol after 43 years of no-tillage or conventional tillage

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ABSTRACT

Occasional strategic tillage (ST) has been suggested as a possible solution to manage herbicide-resistant weeds and control crop diseases in Australia's northern grain-growing regions. We evaluated the impact of ST on microbial communities as indicators of soil quality for two distinct tillage systems that have been applied to a Vertosol for 43 years (no-tillage – NT or conventional tillage – CT) and two stubble management practices (retention – SR or burning – SB). Soil samples were collected 15 weeks after ST and analysed for total enzymatic activity (fluorescein diacetate assay), metabolic diversity (Ecoplates Biolog®) and bacterial community structure (terminal restriction fragment length polymorphism). There was no significant effect of ST on the measured biological attributes. However, total enzymatic activity for treatments under CTSR and CTSR-ST were significantly higher compared with NTSR-ST (+0.8 fluorescein μg ml^{-1} g^{-1} soil h^{-1}, $P < 0.01$). Differences may be attributed to a significant increase in bulk density for CTSR treatment ($P < 0.05$) and an increment in bulk density on CTSR-ST plots. The lack of changes may be attributed to a high resistance and/or resilience of soil microbial communities after 15 weeks of tillage. More studies on the long-term effect of ST are required to assess the impact on soil biological properties.

1. Introduction

Trends in Australian farming have been towards the implementation of conservation agriculture (CA). According to the Food and Agriculture Organization of the United Nations, CA can be achieved by the reduction of soil disturbance, application of crop rotation and increase of soil cover [1]. Agricultural practices such as no-tillage (NT) and introduction of fallow periods during summer in areas where stubble is retained have improved soil water retention, reduced labour costs and increased soil organic matter accumulation [2–4]. Nonetheless, conventional tillage (CT) is still used to prepare seed beds, mix surface and subsurface soils to allow nutrient flow, eliminate weed growth and allow water infiltration [5]. The frequent soil disturbance destroys soil aggregates and leaves the soil surface directly exposed, which accelerates evaporation rates [6]. Moreover, the lack of integrated strategies for weed control and crop residues retention in NT systems have resulted in the escalation of herbicide-resistant weeds and an increase in stubble-borne diseases, especially in wet seasons [7–10]. The use of occasional strategic tillage (ST) has been proposed to manage the specific constraints of NT and CT farming systems. ST is defined as an opportunistic use of an occasional tillage in an otherwise NT system to address specific biotic or abiotic challenges that takes into account the soil water content and time of tillage implementation [11]. Furthermore, ST aims at reducing the disturbance towards soil properties to maintain soil quality. Still, soil management influences soil chemical and physical properties and may affect functions of soil microbial communities and structure that might jeopardise or enhance soil quality [12,13].

Previous studies on the impact of ST in a range of soils under NT have shown that ST has a low impact on soil ecosystem functions and productivity. However, soils with weak A-horizons and contrasting textural properties are more prone to adverse impacts on soil fertility [11,14,15].

Soil microbial communities are considered suitable indicators for soil quality due to rapid responses of microorganisms to disturbance events [16]. The degree of soil disturbance can be measured using different biological indicators including enzymatic and microbial metabolic activity as well as genetic fingerprinting profiles. For instance, enzymatic activity is widely used due to the role of microorganisms in the biochemical functions associated

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with the C, N, S and P cycles [17,18]. Fluorescein Diacetate (FDA) is a broad spectrum detector of enzymatic activity of microbial communities since non-specific esterases, proteases and lipases have been shown to hydrolyse FDA [19]. In a comparison between conservation tillage, reduced tillage and non-inversion tillage, FDA showed higher enzymatic activity (15–40%) in the top 0.15 m soil layer in NT and reduced tillage systems [20].

Measurement of metabolic activity through Community-Level Physiological Profiles (CLPP) provides information on the dynamics and functional adaptation of soil microbial communities [21,22]. Several studies have shown that tillage influenced utilisation of substrates by microbial communities [23–25]. Cookson et al. [26] proposed that these changes may be related to the impact of disturbances in total soil organic matter, light fraction organic matter and dissolved organic matter, all of which play a major role in C and N supply to soil microbial communities. Furthermore, Terminal Restriction Fragment Length Polymorphism (T-RFLP) has been used to study variations between genes from different bacteria and to obtain information on the structure of soil communities [27,28]. In agricultural soils, T-RFLP has been used to compare changes in the structure of bacterial communities in Vertosols and Calcisols under different tillage systems [29–31]. In addition, Girvan, et al. [32] used T-RFLP, denaturing gradient gel electrophoresis and Biolog® to compare different agricultural sites with contrasting soil types. This study concluded that soil type is a key factor determining bacterial community composition in arable soils. Historically, soil biological properties are the least studied within the discipline of soil science as they were often limited by the unavailability of sensitive techniques to identify and quantify soil microorganism in bulk soil and rhizosphere [33].

In this study, we hypothesize that soil health is not affected by occasional chisel tillage, which is commonly used in north-eastern grains growing region of Australia. This practice is implemented when suitable soil water content is achieved. The winter fallow period has been chosen for soil sampling to minimize data variability, which could be increased by additional factors unrelated to tillage associated with the presence of a crop at the site. Total microbial enzymatic and metabolic activity, as well as bacterial community structure via genetic fingerprinting were assessed as biological indicators of soil health. The analysed Vertosol has been under NT or CT and stubble retention (SR) or burning (SB) management for 43 years.

2. Materials and methods

2.1. Site description

The plots were established in December 1968 in a factorial design to study the effect of tillage, crop residue management and nitrogen (N) fertiliser on crop yields at the Hermitage Research Station (28°12′ S, 152°06′ E) in Queensland, Australia. Since their establishment, all plots were monocultured with wheat (*Triticum aestivum* L. cv. Timgalen in the first 29 years and cv. Baxter/cv. Gregory in the remaining years). Rotation with barley (*Hordeum vulgare* L. cv. Clipper) was used for a period of three years (1975–1977) to break the cyclic infection of the fungus *Pyrenophora triticirepentis* [34–36]. The soil is described as black, montmorillonitic, cracking clay, Vertosol [37] with alternating wet-dry conditions and rich in swelling clays. The first 0.1 m contains 650 g kg^{-1} clay, 240 g kg^{-1} silt and 110 g kg^{-1} sand. The mean recorded annual rainfall is 650 mm–700 mm, with more than 50% received during summer [34]. Air temperatures vary from approximately 28 °C in summer to 18.7 °C during winter.

2.2. Soil sampling

Treatments were aligned longitudinally, replicated into four randomised plots of approximately 61.9 m × 6.0 m with 0.8 m buffer between each plot. Original treatments (long-termed, primary) followed a factorial combination of CT and NT, SR and SB crop residue management, as follows: soils under CT were subjected to four to five passes with a chisel plow during the fallow period for weed control. NT treatment involved only seed planting and fertiliser placement. Stubble retention was performed after the crop harvest, whereas stubble burning was applied each year in December or January depending on weather conditions [38,39]. For the purpose of this study, targeted plots have not been treated with N fertiliser (0 kg N ha^{-1} yr^{-1}). March 2012 was chosen as the preferred time to implement ST because it was neither too close to sowing of the winter crop (most preferred time of wheat sowing at this site is mid-June), nor too close to the harvest of the previous crop (November 2011). Poor crop establishment due to decrease in soil moisture was the main concern which prevented the application of ST close to sowing. Likewise, ST immediately after the harvest of the previous crop could have resulted in incorporation of crop residues into the soil and consequently accelerated decomposition, thereby resulting in a loss of soil cover. Historical rainfall data for the site showed that the probability of getting enough rainfall (125 mm) to replenish lost moisture in evaporation with ST was 90–95% for the period between March to June. Each original plot was divided longitudinally to facilitate ST on one side and retained original treatment on the other side, with effective plot size of 61.9 m × 2.7 m, leaving a 0.6 m between original and ST. Therefore, the treatments were NTSR, NTSR-ST, NTSB, NTSB-ST, CTSR, CTSR-ST, CTSB and CTSB-ST. Each treatment included four replications. Soil samples were collected 15 weeks after the ST operation. Five randomised soil samples per plot were collected in separate sealed bags from the top 0.1 m soil depth, using a hand shovel. Soil samples from the same plot were composited, sieved (sieve porosity < 4 mm) and stored at 4 °C for further analysis. All visible litter material was manually removed prior to sieving. For bulk density and chemical analysis, two soil samples from each replicate were taken 3 months after the initial tillage by using a 43 mm-diameter tube sampler attached to hydraulic soil-sampling rig. The first sample from each replicate was oven-dried at 105 °C and the second sample was oven-dried at 40 °C and ground to pass a 2 mm sieve. Bulk density was calculated by taking the mass of oven-dried soil (105 °C) per unit volume of the soil sample, and the volumetric water content was calculated by multiplying the gravimetric water content by the bulk density value from the first sample. The second sample was used to determine total organic carbon (TOC). Total nitrogen (TN) was measured on a subsample from the second replicate and ground to pass through a <0.5-mm sieve [40]. Equivalent soil mass was used to compare TOC stocks [41].

2.3. Fluorescein diacetate (FDA)

Total enzymatic activity was measured using the method developed by Adam and Duncan [42]. In brief, 2 g of fresh soil was incubated with 15 mL of 60 mM potassium phosphate buffer (pH 7.6). To start the reaction, a volume of 200 μL of FDA (1000 μg/mL) was added and incubated for 1 h at 30 °C and 150 rpm in an incubator/shaker (N-biotek. Inc.). After incubation, 1 mL of the mix was added to a new microcentrifuge tube containing 1 mL of 2:1 chloroform-methanol. Triplicates were collected per sample after incubation. The soil suspension was centrifuged for 3 min at 10,000 *g*. Aliquots of 250 μL were transferred into a 96-well plate and read in a microtitre plate reader (BMG Lab, Ortenberg,