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Conservation tillage positively influences the microflora and microfauna in the black soil of Northeast China

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ABSTRACT

Soil food webs are important in maintaining agricultural productivity and ecosystem health. However, our understanding is still limited with respect to the influences of tillage transitions on soil food webs. The present study aimed to quantify the response of microflora and microfauna, and their linkage to different tillage treatments: no tillage (NT), ridge tillage (RT) and conventional tillage (CT). Soil samples were collected from 0 to 20 cm depth in April of 2011 after 10 years of conservation tillage. The abundance and richness of bacteria and arbuscular mycorrhizal fungi were greater in NT and RT than in CT. In case of microfauna also, similar patterns were observed with greater protozoa, bacterivores and omnivores– carnivores in NT and RT compared to CT. The connectance of the bacterial and predator–prey pathways was greater in NT and RT than in CT and that of fungal pathway was greatest in RT. The trophic relationship of the bacterial and predator–prey pathways was strengthened due to the higher water content of soil and the lower $NO₃$ -N after the conversion of CT to NT and RT. Our study suggested that 10 years of conservation tillage can effectively enhance the structure and function of soil food webs through bottom–up effects in the black soil region of Northeast China.

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1. Introduction

Soil food webs deliver important ecosystem services, which are necessary to maintain agricultural productivity and ecosystem health ([Minoshima et al., 2007; Sánchez-Moreno and Ferris, 2007\)](#page-6-0). The abundance and trophic relationship of food web components, including microorganisms (bacteria and fungi), microbivores (protozoa and nematodes) and predators (nematodes) ([Li et al.,](#page-6-0) [2012; Scharroba et al., 2012\)](#page-6-0), highly depend on soil management ([Coleman, 2008](#page-5-0)). Changes in soil food webs can affect mineralization of nutrients and decomposition of organic matter [\(Wardle, 2002; Li](#page-6-0) [et al., 2012\)](#page-6-0). Therefore, understanding the changes in microflora and microfauna, and their linking across contrasting soil managements could lead to a precise regulation of soil organisms for sustainable agroecosystems ([Treonis et al., 2010; Wall et al., 2012\)](#page-6-0).

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Tillage affects soil organisms by changing the soil physical environment and the food supply ([Kladivko, 2001; Kladivko and](#page-6-0) [Clapperton, 2011; Sánchez-Moreno et al., 2011](#page-6-0)). In conventional tillage (CT), soil communities typically consist of bacteria and herbivorous nematodes [\(Govaerts et al., 2006; Kuntz et al., 2013\)](#page-5-0); whereas, the communities in conservation tillage support a high proportion of fungi and predatory nematodes [\(Minoshima et al.,](#page-6-0) [2007; van Capelle et al., 2012; Zhang et al., 2012\)](#page-6-0). Until now, little research has investigated the response of soil microflora and microfauna simultaneously to different tillage treatments.

Conventional tillage in the black soil (Typic Hapludoll, USDA Soil Taxonomy) of Northeast China has beenwidely used in this region for decades. Continuous moldboard plowing and the removal of postharvest residues from the CT have seriously degraded the soil ([Liu et al., 2010](#page-6-0)). Conservation tillage, including no tillage (NT) and ridge tillage (RT), has been proposed to farmers to replace CT in part, but large-scale application of conservation tillage can only be achieved by demonstrating their benefits for soil and plants.

To reveal the relationships between structure and functioning, three food web pathways were compartmented accounting for the main flux of C through the web: (1) the bacterial pathway, in which

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C flows from bacteria to their grazers (protozoa and bacterivorous nematodes); (2) the fungal pathway, in which C flows from fungi to fungivorous nematodes; and (3) the predator–prey pathway, in which C flows from nematode prey (protozoa, microbivorous and herbivorous nematodes) to nematode predators [\(Holtkamp et al.,](#page-6-0) [2008; Sánchez-Moreno et al., 2011; de Vries et al., 2012\)](#page-6-0). We hypothesize that (1) NT and RT, compared with CT, positively affect the components of the food webs, with the fungal-based decomposition pathway dominant; and (2) NT and RT increase the stability and strengthen the trophic relationship of the food webs.

2. Materials and methods

2.1. Experimental site

The tillage experiment was initiated in fall 2001 at the experimental station (44°12′N, 125°33′E) of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences. The station is located in Dehui County, Jilin Province in the northeastern part of China. The mean annual temperature is $4.4\degree$ C, the mean annual precipitation is 520.3 mm with more than 70% of the precipitation between June and August. The soil is classified as black soil (Typic Hapludoll, USDA Soil Taxonomy) with a clay loam texture (the average soil texture was $36.0 g kg^{-1}$ clay, 24.5 g kg⁻¹ silt and 39.5 g kg^{-1} sand). Before the tillage trail initiated, the field has been used for continuous maize (Zea mays L.) with conventional tillage for more than 20 years [\(Liang et al., 2007](#page-6-0)).

2.2. Experimental design

The tillage experiment consisting of no tillage (NT), ridge tillage (RT) and conventional tillage (CT) was arranged in a completely randomized design with a maize–soybean (Glycine max Merr.) rotation system ([Liang et al., 2007](#page-6-0)). Each treatment area having four replications was $5.2 \text{ m} \times 30 \text{ m}$ with 5 m buffer rows between treatments. The crops were sown in May and harvested in October followed by fallow for 6 months. Treatments in the CT consisted of moldboard plowing (20 cm) in the fall followed by secondary seedbed preparation by disked (7.5–10 cm) and harrowed in spring. For the NT, the soil was undisturbed, except when the crop was planted using a KINZE-3000 NT planter (Williamsburg, Iowa). In RT, ridges were maintained year-to-year with a cultivator (John Deere Company, US) in each June, and a modified lister and scrubber was used to form and press the ridge (16 cm in height and 75 cm in width). Maize and soybean were planted with a no-till planter. After harvest, the maize straw in RT and NT was cut into pieces of approximately 30 cm leaving a 30–35 cm stubble stand, and the straw pieces were then returned to the soil surface; soybean residues in RT and NT were directly returned to the soil surface.

The application rates of N, P and K were the same in all the three treatments. Each year, 100 kg N ha $^{-1}$, 45.5 kg P ha $^{-1}$ and 78 kg K ha $^{-1}$ were applied to maize as basal fertilizer. An additional 50 kg N ha^{-1} was applied as a top dressing at the V-6 stage (6 maize leaves with collars). For soybean, all fertilizers were applied as basal fertilizer, including 40 kg N ha $^{-1}$, 60 kg P ha $^{-1}$ and 80 kg K ha $^{-1}$. An attachment to the no-till planter banded the basal fertilizers at planting.

2.3. Soil sampling

Soil samples from each treatment were collected from 0 to 20 cm depth in April of 2011. Each soil sample was pooled from six soil cores of 2.5 cm diameter. In the center of each treatment, bulk density was determined from the surface to 20 cm depth at 5 cm intervals using a 100-cm³ cylinder (5 cm height \times 5 cm diameter). Each sample was split into two subsamples. One was stored at 4° C for <2 weeks for soil biological analysis, and the other was airdried and sieved within one month for physical and chemical analysis.

2.4. Soil physical and chemical properties

Bulk density (BD) was determined using the core method ([Grossman and Reinsch, 2002\)](#page-5-0). Water content of soil (WCS) was measured gravimetrically. Soil pH was determined with a glass electrode in 1:2.5 soil: water solution (w/v). Soil inorganic N (NO₃⁻-N and NH_4^+ -N) was first extracted with 2 M KCl, and then the filtrates were determined using a flow injection auto analyzer (FIAstar 5000 Analyzer, Foss Tecator, Hillerød, Denmark). The concentrations of soil inorganic N were calculated based on dry soil weight. Soil total carbon and total nitrogen (TN) contents were each determined using a FlashEA 1112 elemental analyzer (ThermoFinnigan, Italy). Because the soil was free of carbonate, the soil organic carbon (SOC) was assumed to be equal to the total carbon. The C/N ratio was calculated by dividing SOC by total N.

2.5. PLFA and protozoa analysis

The soil microbial community was characterized using phospholipid fatty acids (PLFAs) analysis as described by [Bossio and](#page-5-0) [Scow \(1998\)](#page-5-0) and [Briar et al. \(2011\)](#page-5-0). Lipids were extracted from 8 g of freeze-dried soil using a chloroform–methanol–citrate buffer mixture (1:2:0.8). The polar lipids were separated from neutral lipids and glycolipids on a solid phase extraction columns (Supelco Inc., Bellefonte, PA). The phospholipids were trans-esterified to a mild-alkali methanolysis and the resulting fatty acid methyl esters were extracted in hexane and dried under N_2 . Samples were then dissolved in hexane and analyzed in an Agilent 6850 series Gas Chromatograph with MIDI peak identification software (Version 4.5; MIDI Inc., Newark, DE).

The following biomarkers were used: Total PLFA (sum of all identified PLFAs; from C14 to C20); gram-negative bacteria $(16:1\omega$ 7c, cy17:0, 16:1 ω 9c, 17:1 ω 8c, 18:1 ω 7c, cy19:0, 16:1 2OH); gram-positive bacteria (i14:0, i15:1, i15:0, a15:0, i16:0, i17:0, a17:0) ([Aciego Pietri and Brookes, 2009; Bach et al., 2010\)](#page-5-0); saprophytic fungi (18:1 ω 9c and 18:2 ω 6c) ([Li et al., 2012; Dempsey et al., 2013\)](#page-6-0); and arbuscular mycorrhizal fungi (AMF) (16:1 ω 5c) ([McKinley et al.,](#page-6-0) [2005; Bach et al., 2010\)](#page-6-0). The sum of the gram-negative bacteria, gram-positive bacteria and non-specific bacteria (14:0, 15:0, 16:0, 18:0, 20:0) was expressed as the total bacteria. The PLFA richness was calculated as the number of different PLFAs detected per sample, and abundance was expressed in nmol g^{-1} dry soil.

The most-probable-number method was used to determine flagellate populations ([Singh, 1975; Rodriguez-Zaragoza et al.,](#page-6-0) [2005; Li et al., 2012](#page-6-0)). The assays were performed in 24-well cell culture plates and the growth medium in each well was 0.9 mL autoclaved and filtered soil extract (1:5, soil:water). The first well of each dilution series was inoculated with a 0.1 mL aliquot of 1:10 soil suspension shaken in a vortex for five 15-s pulses. Four replicates 10-fold dilutions to 10^{-7} were prepared for each soil sample. The plates were incubated at $28\degree C$ for 7-10 days and reviewed with an inverted microscope for the presence of flagellates. Abundance and richness of flagellates were expressed as the number of individuals or taxa per gram of dry soil.

2.6. Nematode determination

Nematodes were extracted from a 50g soil sample (fresh weight) by a modified cotton–wool filter method [\(Liang et al.,](#page-6-0) [2009](#page-6-0)). After counting the total number of nematodes, 100 specimens per sample were randomly selected and identified to the

WCS: water content of soil; BD: bulk density; SOC: soil organic carbon; TN: total nitrogen; C/N: the ratio of SOC to TN; NO₃⁻-N: nitrate N; NH₄⁺-N: ammonium N. Values followed with different superscript letters within a column are significantly different at $P < 0.05$.

genus level. If the total number was less than 100, all of the nematodes were identified. The nematodes were assigned to the following trophic groups characterized by feeding habits: (1) bacterivores (Ba); (2) fungivores (Fu); (3) omnivores–carnivores $(Om + Ca)$; and (4) herbivores (H) according to Yeates et al. (1993) . Abundance and richness of nematodes were expressed as the number of individuals or taxa per 100 g dry soil.

2.7. Statistical analyses

Nematode, protozoan and microbial abundances were $\ln(x+1)$ transformed prior to statistical analysis for normality of data. To assess differences in soil properties and biota among tillage treatments, a one-way ANOVA was performed and means were compared by least significant difference (LSD). A difference at $P < 0.05$ level was considered to be statistically significant. All statistical analyses were performed by SPSS statistical software (SPSS Inc., Chicago, IL). Principal component analysis (PCA) and canonical correspondence analysis (CCA) were performed to explore the composition of the soil biotic community based on the relative abundances of PLFAs and nematodes' data and the relationship between soil biota and environmental parameters using CANOCO software, version 4.5 [\(ter Braak, 1988](#page-6-0)).

To quantify food web stability, the connectance of each pathway was calculated. Connectance (C) is calculated as: $C = L/S^2$, where L is the total number of feeding links in a pathway and S is the number of taxa in a pathway [\(Beckerman et al., 2006; Sánchez-Moreno](#page-5-0) [et al., 2011](#page-5-0)). Little is known of how these soil organisms are linked; it is assumed that any organism in the resource group may be eaten by any organism in the consumer group [\(Sánchez-Moreno et al.,](#page-6-0) [2011\)](#page-6-0).

Structural equation modeling (SEM) was used to investigate how trophic relationships respond to the conversion of CT to conservation tillage. An a priori model was developed based on a literature review and our knowledge of how these predicators are related. In this model, soil properties and bacterial grazers were treated as latent variables. WCS and $NO₃⁻-N$ were treated as the indicators of soil properties, and the abundances of bacterivores and protozoa were treated as the indicators of bacterial grazers. The analysis was conducted using AMOS 7.0 software ([Arbuckle,](#page-5-0) [2006](#page-5-0)). Several tests were used to assess model fit, i.e., the χ^2 -test, comparative fit index (CFI), goodness-of-fit (GFI) and root mean square error of approximation (RMSEM).

3. Results

3.1. Soil physical and chemical properties

Most soil parameters were affected by different tillage treatments after 10 years. NT and RT, compared with CT, increased WCS and C/N and decreased the concentrations of $NO₃$ ⁻-N ($P < 0.05$). SOC was higher in RT than in CT ($P < 0.05$) (Table 1).

3.2. Soil microbial communities

Effects of tillage on soil microbial functional groups were more obvious than on the total PLFAs (Table 2). The abundance of total bacteria, gram-negative bacteria and AMF increased more

Table 2

Abundance and richness of PLFAs and protozoa under no tillage (NT), ridge tillage (RT) and conventional tillage (CT) treatments ($n = 12$).

Total PLFAs: total phospholipid fatty acids; G (+): gram-positive bacteria; G (-): gram-negative bacteria; Sf: saprophytic fungi; AMF: arbuscular mycorrhizal fungi. Values followed with different lowercase letters within a row are significantly different at $P < 0.05$.

Fig. 1. Principle components analysis (PCA) of soil microbial and nematode communities under different tillage treatments (\cap , \triangle , and \Box represent NT, RT and CT, respectively; Ba, bacterivores; Fu, fungivores; H, herbivores; Om + Ca, omnivores–carnivores; G (+), gram-positive bacteria; G (-), gram-negative bacteria; Sf, saprophytic fungi; AMF, arbuscular mycorrhizal fungi).

obviously in NT and RT than in CT ($P < 0.05$). A similar trend was also found in the richness of bacteria and gram-negative bacteria, in which larger values were observed in NT and RT than in CT $(P< 0.05)$ [\(Table 2](#page-2-0)).

Profiles and compositions of microbial communities varied among tillage treatments along PC1, which accounted for 65.8% of the total variation (Fig. 1). Samples in NT and RT were dominated by AMF and gram-negative bacteria; whereas, samples in CT were dominated by saprophytic fungi and gram-positive bacteria. The abundance of flagellates rather than the taxa richness was more sensitive to tillage effect, and higher values were presented in NT and RT in comparison with CT $(P < 0.05)$ [\(Table 2](#page-2-0)).

3.3. Soil nematode communities

The abundance and richness of total nematodes and different trophic groups varied among different tillage treatments (Table 3). Compared with CT, NT increased the abundance of total nematodes, bacterivores and omnivores–carnivores, and RT only increased omnivores–carnivores ($P < 0.05$). The richness of total nematodes was higher in NT than in CT $(P < 0.05)$. Similar observations were also found in bacterivores and omnivores– carnivores. The richness of fungivores increased in RT relative to CT $(P < 0.05)$ (Table 3).

The PCA of nematode community also distinguished CT from NT and RT along PC1 (Fig. 1), with bacterivores and omnivores– carnivores dominant in NT and RT and herbivores prevailing in CT (Fig. 1).

3.4. Associations between soil biota and soil parameters

The CCA analysis suggested that the first two axes explained 99.8% and 78.1% of the total variations in soil microorganisms and nematodes, respectively ([Fig. 2](#page-4-0)). WCS and $NO₃⁻-N$ were the most important contributors to the distribution of microbial and nematode communities ([Fig. 2](#page-4-0)).

3.5. Soil food webs

The connectedness within the soil food webs differed among tillage treatments ([Fig. 3](#page-4-0)). The connectance of bacterial and predator–prey pathways was significantly higher in NT and RT than in CT and that of fungal pathway was greatest in RT ($P < 0.05$). Trophic relationships for the bacterial and predator–prey pathways were strengthened and highly associated with soil properties after the conversion of CT to conservation tillage [\(Fig. 4](#page-5-0)).

4. Discussion

4.1. Effects of tillage on soil microbial and nematode communities

In this study, as expected, the abundance and richness of primary decomposers, such as total bacteria, gram-negative bacteria and AMF, were greater in NT and RT than in CT ([Table 2](#page-2-0)). Similar results were also found in semi-arid and marine climate conditions [\(Helgason et al., 2010; Kuntz et al., 2013\)](#page-6-0). This may be due to the increased input of crop residues in NT and RT, which can serve as food resources for microorganisms ([Scheu and Schaefer,](#page-6-0) [1998](#page-6-0)). Moreover, the composition of soil microbial communities was also altered by the different tillage treatments, with a shift from gram-positive bacteria and saprophytic fungi dominant in CT to gram-negative bacteria and AMF dominant in NT and RT (Fig. 1). The close association between gram-positive bacteria and CT was also reported by [Zhang et al. \(2005\)](#page-6-0) and [Kennedy and Schillinger](#page-6-0) [\(2006\)](#page-6-0) in the loamy texture soils. This finding may be explained by the changes in the quality of organic matter across different tillage treatments ([Wang et al., 2012](#page-6-0)) because the high proportion of gram-negative bacteria usually indicates a shift from oligotrophic to more copiotrophic conditions in the soil ([Kourtev et al., 2003;](#page-6-0) [Zhong et al., 2010](#page-6-0)). Consequently, the higher value of C/N in NT and

Table 3

Abundance and richness of nematode communities under no tillage (NT), ridge tillage (RT) and conventional tillage (CT) treatments ($n = 12$).

Ba: bacterivores, Fu: fungivores, H: herbivores, Om + Ca: omnivores–carnivores. Values followed with different lowercase letters within a row are significantly different at $P < 0.05$

Fig. 2. Canonical correspondence analysis (CCA) showing the relationship between soil biota ((a) microbial community; (b) nematode community) and soil parameters. WCS, water content of soil; BD, bulk density; SOC, soil organic carbon; TN, total nitrogen; C/N, the ratio of SOC to TN; NO_3 ⁻-N, nitrate N; NH_4 ⁺-N, ammonium N. G $(+)$, gram-positive bacteria; G $(-)$, gram-negative bacteria; Sf, saprophytic fungi; AMF, arbuscular mycorrhizal fungi; Ba, bacterivores; Fu, fungivores; H, herbivores; Om + Ca, omnivores–carnivores.

RT [\(Table 1](#page-2-0)) has the potential to increase gram-negative bacteria in comparison with CT. Although physical disruption is considered specifically detrimental to fungi, the response of different fungal species to tillage may differ [\(Calderón et al., 2001\)](#page-5-0). Saprophytic fungi are believed to be less susceptible to tillage stress than AMF ([Wortmann et al., 2008; van Groenigen et al., 2010\)](#page-6-0), therefore, saprophytic fungi relative to AMF were particularly dominant in CT ([Fig. 1](#page-3-0)).

In addition, higher abundances of protozoa and bacterivores in NT were related to the increase in bacteria [\(Fu et al., 2000; Briar](#page-5-0) [et al., 2007; Rønn et al., 2012\)](#page-5-0). The lower predator biomass in CT

Fig. 3. The connectance of bacterial, fungal and predator-prey pathways under different tillage treatments (bars represent the standard error). Capital letters indicate significant differences among tillage treatments.

(Supplementray Table S1) can partially explain the greater proportion of herbivores compared to the NT and RT ([Fig. 1\)](#page-3-0). Our results were consistent with the findings of [Govaerts et al.](#page-5-0) [\(2006\)](#page-5-0) and [Okada and Harada \(2007\)](#page-6-0) after 6 years of NT. The relatively higher abundance of omnivores–carnivores in NT and RT ([Fig. 1](#page-3-0)), compared with CT, indicates that soil food webs in conservation tillage may offer biological buffering capacity and prevent individual organisms (i.e., nematode pests) from becoming dominant through predation ([Yeates and Wardle, 1996; DuPont](#page-6-0) [et al., 2009; Zhang et al., 2013\)](#page-6-0).

4.2. Effects of tillage on the structure of soil food webs

Connectance represents the stability of the food web network ([Dunne et al., 2004; Beckerman et al., 2006](#page-5-0)). The connectance of the bacterial and predator–prey pathways was higher in NT and RT than in CT (Fig. 3), and the connectance of fungal pathway was greatest in RT. This finding suggests that NT and RT, in comparison with CT, can build more complex interactions between consumers (i.e., bacterivores) and resources (i.e., bacteria). Our observations indicate that the stability of the food webs was enhanced by 10 years of conservation tillage.

The SEM analysis in this study supported the bottom–up effects of tillage on soil organisms [\(Fig. 4\)](#page-5-0). The general concept is that when conservation tillage is operated over an 8-year period, the pathway of organic matter decomposition is predominantly

Fig. 4. Structural equation model showing the feeding relationship in the food web in response to the conversion from conventional tillage to conservation tillage $(\chi^2 = 19.361, df = 23, P = 0.680, CF = 1.000, GF = 0.824, RMSEA = 0.000)$. Solid line arrows represent significant paths $(P < 0.05)$, and dotted lines indicate nonsignificance. Arrow thickness represents the magnitude of the path coefficient. WCS, water content of soil; $NO₃$ ⁻-N, nitrate N.

mediated by the fungi (Adl et al., 2006; Bailey et al., 2002; Griffiths et al., 2012). In the present study, although the stability of the fungal pathway was enhanced by RT [\(Fig. 3\)](#page-4-0), there was no shift in the composition of soil communities from bacterial- to fungalbased food webs after 10 years of conservation tillage (Fig. 4). Two explanations are possible. First, this might be due to differences in resilience of the soil food webs. Compared with the bacterial-based food web, the fungal-based food web is less resilient with relatively lower recovery rate after disturbance (de Vries et al., 2012). Similarly, [Liiri et al. \(2012\)](#page-6-0) also claimed that intensive land use could have a long-lasting effect on soil ecosystem. Secondly, the other functional groups, such as Mites and Collembola, are also major fungal feeders ([Holtkamp et al., 2008; Ngosong et al., 2009\)](#page-6-0). In our study only nematodes were included in the fungivorous fauna. Thus, investigating certain microarthropod groups is critical for further understanding the food webs response to tillage treatments in the black soil of Northeast China.

The responses of the soil food webs to different tillage may depend on changes in the soil microenvironment, which provides feedback that regulates the structure of the food webs ([Hedlund](#page-6-0) [et al., 2004; Scharroba et al., 2012](#page-6-0)). The SEM analysis highlighted that soil food web structure was closely correlated with the WCS and $NO₃$ ⁻-N (Fig. 4). These results suggest that the higher WCS and the lower $NO₃$ ⁻-N in NT and RT can strengthen the trophic relationships of the bacterial and predator–prey pathways. Our observation was partially consistent with the findings of Culman et al. (2010) and [Sánchez-Moreno et al. \(2011\)](#page-6-0), who found that $NO₃$ ⁻-N, an indicator of perturbation intensity, was negatively correlated with biological diversity.

5. Conclusions

Our results partially supported our hypothesis that the abundance and richness of most microflora and microfauna are positively influenced by NT and RT. The stability and trophic links of bacterial and predator–prey pathways were strengthened in NT and RT compared to CT. This study suggests that a more functionally stable food web can be built through the bottom– up effects after 10 years of conservation tillage.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [http://dx.doi.org/10.1016/j.still.2015.01.001.](http://dx.doi.org/10.1016/j.still.2015.01.001)

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