LAYANE APARECIDA MENDES DOS SANTOS

CONTRIBUIÇÃO DA MICROBIOTA DO SOLO NO CRESCIMENTO DO ALGODOEIRO EM SISTEMAS CONSOLIDADOS E RECÉM-IMPLANTADOS DE SUCESSÃO SOJA/ALGODÃO

LAVRAS – MG
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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Biologia, Microbiologia e Processos Biológicos do Solo, para obtenção do título de Mestre.

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CONTRIBUTION OF SOIL MICROBIOTA TO COTTON GROWTH IN CONSOLIDATED AND NEWLY IMPLEMENTED SOYBEAN/COTTON SUCCESSION SYSTEMS

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração Biologia, Microbiologia e Processos Biológicos do Solo, para obtenção do título de Mestre.

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À minha pequena grande família, que sonhou comigo.
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RESUMO GERAL

Agricultores relatam redução do crescimento e produtividade do algodão ao substituir inicialmente o milho na sucessão soja/milho, mesmo sob condições químicas e físicas do solo adequadas. A biologia do solo das áreas consolidadas de soja/algodão, pode estar atuando na disponibilidade e absorção de nutrientes. O objetivo do estudo foi de investigar o efeito do cultivo sucessivo do sistema soja/algodão sobre a disponibilidade e absorção de nutrientes, condições microbiológicas do solo e crescimento inicial do algodoeiro. Foram realizados dois experimentos em casa de vegetação, sendo: (1) tempo de cultivo e (2) diluição/autoclavagem, o solo de ambos foram coletados em Sapezal-MT. O experimento (1) foi realizado em delineamento inteiramente ao acaso (DIC), sendo 5 tratamentos com solo de diferentes históricos/tempo de cultivo de soja/algodão (T0 - cultivo consolidado de soja/milho; T1 - primeiro ano soja/algodão; T2 - segundo ano soja/algodão; T3 - terceiro ano soja/algodão e T4 – quarto ano soja/algodão), com três repetições. O experimento (2) foi realizado em DIC com esquema fatorial (4 × 2), com solo de cultivo consolidado de soja/algodão (≥10 anos). Quatro proporções de solo (25, 50, 75 e 100%) em relação à areia autoclavada e duas condições microbiológicas (SA – solo autoclavado e SNA – não-autoclavado), com três repetições. A unidade experimental foi composta por um vaso, sendo: (1) um litro e (2) dois litros. Após 30 dias (1) e 40 dias (2) foram avaliadas: massa seca da parte aérea (MSPA), raízes (MSR) e total (MST); concentração e acúmulo de nutrientes foliar; teor de nutrientes e fracionamento de P no solo; biomassa microbiana e bioquímica do solo, colonização radicular por fungos micorrízicos arbusculares (FMA) e nível crítico de P para o algodoeiro (NC). Os dados foram submetidos à análise de variância, teste de Tukey e correlação de Pearson. Não houve efeito ($p \geq 0,05$) no crescimento das plantas no experimento (1), porém, houve aumento na concentração e acúmulo de P e FMA em função do aumento do tempo de cultivo da soja/algodão. O experimento (2) também aumentou de P foliar e FMA devido à preservação microbiológica, além disso, aumento de 36 e 58% na produção de MSPA e MSR nos solos 100% SNA, respectivamente, quando comparado com 100% SA. Quanto aos FMA, o tratamento T4 no (1) obteve aumento de 49%, 53%, 82% e 10% em relação ao T0, T1, T2 e T3, respectivamente e o (2) apresentou média de 84% e 2% para SA e SNA, respectivamente. No tratamento 100% SA do (2), o teor de P foi três vezes menor que o 100% SNA, ficando abaixo do NC para o algodão, e no (1) apenas o T4 está dentro do NC. O tratamento 100% SNA aumentou 41% no P lábil (extrator Mehlich-3) comparado ao 100% SA e o tratamento T4 aumento de 79, 64, 81 e 32% comparado ao T0, T1, T2, T3 e T4, respectivamente. Conclui-se que o crescimento das plantas em sistemas consolidados de soja/algodão se deve, em parte, à ação de microrganismos na labilidade do P e à melhor absorção por fungos micorrízicos arbusculares.

Palavras-chave: fungos micorrízicos arbusculares. fósforo. sistemas de produção. microbioma.

GENERAL ABSTRACT

Farmers report reduced cotton growth and productivity when initially replacing corn in the soy/corn succession, even under adequate chemical and physical soil conditions. The biology of the soil in consolidated soybean/cotton areas may be influencing the availability and absorption of nutrients. The objective of the study was to investigate the effect of successive cultivation of the soybean/cotton system on the availability and absorption of nutrients, microbiological conditions of the soil and initial growth of the cotton plant. Two experiments were carried out in a greenhouse, namely: (1) cultivation time and (2) dilution/autoclaving, the soil from both were collected in Sapezal-MT. The experiment (1) was carried out in a completely randomized design (CRD), with 5 treatments with soil from different histories/time of soybean/cotton cultivation (T0 - consolidated soybean/corn cultivation; T1 - first year soybean/cotton; T2 - second year soybean/cotton; T3 - third year soybean/cotton and T4 – fourth year soybean/cotton), with three repetitions. Experiment (2) was carried out in CRD with a factorial scheme (4 × 2), with soil from consolidated soybean/cotton cultivation (≥10 years). Four proportions of soil (25, 50, 75 and 100%) in relation to autoclaved sand and two microbiological conditions (AS – autoclaved soil and NAS – non-autoclaved), with three replications. The experimental unit consisted of a vessel, being: (1) one liter and (2) two liters. After 30 days (1) and 40 days (2) the following were evaluated: dry mass of the shoot (SDM), roots (RDM) and total (TDM); concentration and accumulation of foliar nutrients; nutrient content and P fractionation in the soil; microbial biomass and soil biochemistry, root colonization by arbuscular mycorrhizal fungi (AMF) and critical P level for cotton (CL). The data were subjected to analysis of variance, Tukey test and Pearson correlation. There was no effect ($p \geq 0.05$) on plant growth in experiment (1), however, there was an increase in the concentration and accumulation of P and AMF due to the increase in soybean/cotton cultivation time. Experiment (2) also increased leaf P and AMF due to microbiological preservation, in addition, a 36 and 58% increase in SDM and RDM production in 100% NAS soils, respectively, when compared to 100% AS. As for AMF, treatment T4 in (1) obtained an increase of 49%, 53%, 82% and 10% in relation to T0, T1, T2 and T3, respectively and (2) presented an average of 84% and 2% for AS and NAS, respectively. In the 100% AS treatment of (2), the P content was three times lower than the 100% NAS, remaining below the CL for cotton, and in (1) only T4 is within the CL. The 100% NAS treatment increased 41% in labile P (Mehlich-3 extractor) compared to 100% AS and the T4 treatment increased 79, 64, 81 and 32% compared to T0, T1, T2, T3 and T4, respectively. It is concluded that plant growth in consolidated soybean/cotton systems is due, in part, to the action of microorganisms on P lability and better absorption by arbuscular mycorrhizal fungi.

Key words: arbuscular mycorrhizal fungi. phosphor. production systems. microbiome.

INDICADORES DE IMPACTO

O objetivo do trabalho foi investigar o efeito do cultivo sucessivo do sistema soja/algodão na microbiota do solo e relacionar com a disponibilidade e absorção de nutrientes no crescimento inicial de plantas de algodão. O objetivo foi traçado devido ao fato de que no Brasil a substituição inicial do milho pelo algodão no sistema soja/milho em diferentes regiões do estado de Mato-Grosso, tem impacto negativo ao crescimento e produtividade do algodão mesmo sob condições químicas e físicas consideradas adequadas, recuperando-se apenas ao longo do cultivo sucessivo de soja/algodão. De fato, o trabalho comprova que há um impacto direto e indireto da microbiota do solo no cultivo do algodoeiro. O cultivo sucessivo de soja/milho estimula uma comunidade microbiana do solo, em especial fungos micorrizicos arbusculares (AMF), que não é favorável ao algodoeiro. Com isso, o cultivo inicial do algodoeiro é comprometido, mas ao longo do tempo através do recrutamento de microrganismos estimulados principalmente pelos exsudatos radiculares do algodão, ao longo do tempo, mais precisamente aos 4 anos do sistema soja/algodão tem-se um microbioma do solo favorável ao algodoeiro. Esse novo microbioma do solo favorece o microbioma associado a rizosfera do algodoeiro, contribuindo com processos biológicos do solo, como por exemplo a simbiose mutualística com FMA, melhorando principalmente o acesso de nutrientes como o fósforo (P). Sabe-se da difícil dinâmica de P no solo, principalmente em solos brasileiros devido a retenção (adsorção) do mesmo nos solos. Este trabalho traz consigo a importância da microbiota do solo principalmente aos sistemas de produção de cultivo atuais, que são cada vez mais pobres e intensivos, demostrando a necessidade de novos manejos que estimulem a microbiota do solo, além disso, permite e estimula novas pesquisas para melhor refinamento e identificação dos microrganismos benéficos ao algodão. Os resultados do trabalho geram impactos sociais e econômicos. Os impactos sociais do trabalho é a evidência da necessidade de boas práticas de manejo para melhoria e manutenção da qualidade do solo, visando sustentabilidade e qualidade do sistema produção e impactos indiretos a saúde humana, contribuindo com os objetivos da ONU como fome zero e agricultura sustentável, consumo e produção responsáveis e ação contra a mudança global do clima. Além disso, a demonstração de como FMA associados ao algodão favorecem melhor o acesso e eficiência de uso de P, visto pelo incremento de 48% de acúmulo de P nas plantas em tratamentos com maior tempo de cultivo em sistema soja/algodão e maior colonização micorrízica das raízes. Esse melhor aproveitamento de, favorece a otimização desse nutriente grandemente importado. O impacto econômico gerado atende o objetivo da ONU quanto ao trabalho decente e crescimento econômico, os resultados indicam que o problema das áreas reladas por diversos produtores rurais do Mato-Grosso é biológico/microbiano, com este indicativo é possível reavaliar/reformular manejos que melhorem a produtividade do algodoeiro em soja/algodão, sendo incialmente inferior a 150@ de algodão em caroço quando comparado ao sistema consolidado com > 400@ de algodão em caroço. Isso contribuindo para uma produção sustentável e economicamente viável.

IMPACT INDICATORS

The objective of the work was to investigate the effect of successive cultivation of the soybean/cotton system on the soil microbiota and relate it to the availability and absorption of nutrients in the initial growth of cotton plants. The objective was set due to the fact that in Brazil the initial replacement of corn by cotton in the soy/corn system in different regions of the state of Mato-Grosso, has a negative impact on the growth and productivity of cotton even under chemical and physical conditions considered adequate, recovering only during successive soybean/cotton cultivation. In fact, the work proves that there is a direct and indirect impact of the soil microbiota on cotton cultivation. Successive soybean/corn cultivation stimulates a soil microbial community, especially arbuscular mycorrhizal fungi (AMF), which is not favorable to cotton. As a result, the initial cultivation of the cotton plant is compromised, but over time through the recruitment of microorganisms stimulated mainly by cotton root exudates, over time, more precisely after 4 years of the soy/cotton system, there is a microbiome of the soil favorable to cotton. This new soil microbiome favors the microbiome associated with the cotton rhizosphere, contributing to biological processes in the soil, such as mutualistic symbiosis with AMF, mainly improving the access of nutrients such as phosphorus (P). It is known about the difficult dynamics of P in the soil, especially in Brazilian soils due to its retention (adsorption) in the soil. This work brings with it the importance of the soil microbiota, especially in current crop production systems, which are increasingly poor and intensive, demonstrating the need for new management that stimulates the soil microbiota, in addition, it allows and stimulates new research to better refinement and identification of microorganisms beneficial to cotton. The results of the work generate social and economic impacts. The social impacts of work are evidence of the need for good management practices to improve and maintain soil quality, aiming at sustainability and quality of the production system and indirect impacts on human health, contributing to UN objectives such as zero hunger and sustainable agriculture, responsible consumption and production and action against global climate change. Furthermore, the demonstration of how AMF associated with cotton favors better access and use efficiency of P, seen by the 48% increase in P accumulation in plants in treatments with longer cultivation time in a soy/cotton system and greater mycorrhizal colonization of the roots. This better use favors the optimization of this largely imported nutrient. The economic impact generated meets the UN objective regarding decent work and economic growth, the results indicate that the problem in the areas reported by several rural producers in Mato-Grosso is biological/microbial, with this indication it is possible to re-evaluate/reformulate management that improves the productivity of the cotton plant in soybeans/cotton, initially being less than 150% of seed cotton when compared to the consolidated system with > 400% of seed cotton. This contributes to sustainable and economically viable production.

SUMÁRIO

PRIMEIRA PARTE

1. INTRODUÇÃO GERAL

O algodão (*Gossypium hirsutum* L.) é uma cultura mundialmente difundida, cultivada em grande escala principalmente em países como China, Índia, Estados Unidos e Brasil (ABRAPA, 2024). Tal importância se dá principalmente mediante a obtenção de fibras que são destinadas a indústrias têxtil, papel e celulose, dentre diferentes outros coprodutos, além do caroço que pode ser utilizado na alimentação animal, produção de óleo e biodiesel (STONEX, 2022).

No Brasil as regiões do Cerrado são grandes produtoras de algodão (HOFFMAN et al., 2020), com forte expressão principalmente no estado do Mato Grosso. O Brasil é mundialmente conhecido pela produção de grãos (soja e milho) em sistema de safras duplas (sucessão de culturas), com isso, nos últimos anos sojicultores e cotonicultores tem investido no sistema soja/algodão, em que o algodão entra substituindo o milho no sistema soja/milho (ALVES et al., 2018). Porém, tem sido relatado que essa substituição incialmente compromete negativamente o crescimento e produtividade do algodão (redução média de 150 arrobas de algodão em caroço) mesmo sob condições químicas e físicas de solo consideradas adequadas (sem informações biológicas), recuperando-se apenas ao longo do cultivo sucessivo soja/algodão.

Ao olhar para a comunidade microbiana, dentre os diversos fatores que podem alterar sua composição, tem-se o componente planta (LUNDBERG et al., 2012; MARQUES et al., 2014). O sistema radicular das plantas é capaz de modular a estrutura da comunidade microbiana associada a rizosfera, devido a liberação de exsudatos, com isso, determinados cultivos prolongados podem conferir redução da diversidade e aumento de grupos microbianos específicos (MOREIRA; SIQUEIRA, 2006).

O trabalho de Qiao et al. (2017) com diferentes tipos de solos e genótipos de algodão apresentou o tal comportamento de redução gradual de alguns grupos microbianos e aumento da abundância de outros promovidos pelo cultivo do algodão. Isso pode promover o desenvolvimento de uma comunidade microbiana adaptada a cultura do algodão e que contribui para seu crescimento e desenvolvimento.

Os microrganismos participam de diversos processos biológicos como decomposição, mineralização de nutrientes, transformação de nutrientes, estruturação do solo, solubilização de nutrientes (aumento da labilidade), além disso, simbioses radiculares como por exemplo fungos

micorrízicos arbusculares (FMA) que melhoram o acesso dos nutrientes pelas plantas (MOREIRA; SIQUEIRA, 2006; DORTZBACH et al., 2013). O algodoeiro tem forte simbiose mutualística radicular com os FMA, favorecendo o desenvolvimento da cultura principalmente pelo aumento da absorção de água e nutrientes pouco móveis como por exemplo o fósforo (P) (WANG et al., 2017). As hifas fúngicas podem armazenar maiores quantidades de fosfatos, armazenando-os e transferindo continuamente as plantas (LIMA, 2020).

A hipótese testada neste estudo foi que o cultivo sucessivo do sistema soja/algodão prolongado modifica propriedades químicas e microbiológicas do solo aumentando o crescimento do algodoeiro, ao contrário da introdução do algodoeiro em substituição do milho no sistema soja/milho. Nesse contexto, o objetivo geral do estudo foi de investigar o efeito do cultivo sucessivo do sistema soja/algodão na microbiota do solo e relacionar com a disponibilidade e absorção de nutrientes no crescimento inicial da planta de algodão.

2. REFERENCIAL TEÓRICO

2.1 Cultura do algodão

O algodão (*Gossypium hirsutum* L. subsp. *latifolium*) é uma planta pertencente à família das malváceas e nativa da América Central (D'EECKENBRUGGE; LACAPE, 2014). Essa espécie possui hábito de crescimento indeterminado, com isso apresenta diferentes fases ocorrendo simultaneamente ao crescimento vegetativo, como aparecimento de gemas reprodutivas (botões florais), florescimento (flores), crescimento e maturação dos frutos (maçãs e capulhos) (ROSOLEM, 2007). O algodão é cultivado comercialmente mediante a obtenção de fibras e caroços, sendo a fibra matéria-prima para indústrias têxtil, produtos de enfermagem e papel e celulose, enquanto o caroço utilizado para obtenção de óleo e biodiesel, alimentação de animais ruminantes e adubação de plantas (STONEX, 2022).

Atualmente, o algodão está entre as mais importantes culturas no mundo, produzida por mais de 60 países dentre os 5 continentes, com média nos últimos anos de 35 milhões de hectares cultivados em todo mundo. O Brasil ocupa quarta posição no ranking mundial de produção de algodão, abrangendo aproximadamente área produtiva de 1.658 mil hectares em todo país e, nesta última safra (22/23) obteve produção média de 3.030 mil toneladas de algodão em pluma (ABRAPA, 2024).

O Brasil também é mundialmente conhecido pela produção de grãos (soja e milho), principalmente em sistema de safras duplas (sucessão de culturas). O estado do Mato Grosso, maior produtor do país, apresenta alta predominância com soja primeira safra, seguida pelo cultivo de milho segunda safra. Contudo, nos últimos anos sojicultores e cotonicultores dessa região têm investido no sistema soja/algodão, ou seja, soja primeira safra seguido de algodão segunda safra, sendo um sistema único em termos de agricultura mundial (ALVES et al., 2018).

O algodoeiro é uma planta rigorosa em termos de qualidade do solo, sendo muito exigente na fertilidade do solo (DORAHY; ROCHESTER; BLAIR, 2004), sensível ao excesso de alumínio (PIVETTA et al., 2019), desenvolvendo potencial máximo produtivo em solos ricos em matéria orgânica e bem estruturados (FREIRE, 2015). A produtividade do algodoeiro responde positivamente a disponibilidade de nutrientes, principalmente P, com isso, os baixos níveis de P no solo afetam crescimento e qualidade da produtividade do algodoeiro (SUN et al., 2023).

Ao retratar os solos de regiões do Cerrado, altamente intemperizados (SANTOS et al., 2018) e com baixas concentrações de P biodisponíveis, a fertilização química torna-se uma prática de baixa eficiência (em média aproveitamento de apenas 30% pelas culturas) (RANIRO et al., 2023). Isso devido à presença abundante de argilominerais de baixa atividade, resultando em forte adsorção de P (ROY et al. 2016), possibilitando que este possa tornar-se fator limitante para o cultivo do algodoeiro.

Um ponto importante é que microbiota do solo participa fortemente de processos biológicos, tais como mineralização e simbioses radiculares que melhoram a disponibilidade de nutrientes no solo. O cultivo do algodoeiro é capaz de estimular determinados grupos de microrganismos do solo que podem ter impacto no seu crescimento, a partir de processos biológicos. Diante disso é necessário avaliar a contribuição da microbiota do solo estimulada pelo algodoeiro em sistema soja/algodão ao longo do cultivo sucessivo, principalmente grupos de microrganismos associados, na melhoria da disponibilidade e absorção de nutrientes.

2.2 Microbioma associado a rizosfera

A rizosfera é definida desde a região dos tecidos corticais das raízes (rizoplano), até uma camada de solo adjacente ao rizoplano (aproximadamente 1 a 3 mm) (PHILIPPOT ET al., 2013) sendo fortemente influenciada pelo metabolismo vegetal por meio da liberação de fotossintatos como uma matriz de exsudatos, secreções, mucilagens, mucigel, lisados, além da liberação de dióxido de carbono (CO_2). Vale ressaltar que esta zona de interação solo-planta constitui um

ambiente altamente favorável à manutenção de uma alta comunidade microbiana (AHKAMI et al., 2017).

O ambiente rizosférico, ao receber tantas substâncias secretadas pela raiz, é capaz de aumentar e/ou alterar a estrutura da comunidade do microbioma da rizosfera (CHEN et al., 2016), que consequentemente favorece processos biológicos como decomposição, mineralização e solubilização de nutrientes; tolerância a estresses abióticos; proteção natural da planta quanto a agentes patogênicos; dentre outros (DUBEY et al., 2019; MOREIRA; SIQUEIRA, 2006).

Grande parte da funcionalidade de determinado microbioma do solo é referente a grupos específicos, os quais possuem determinadas características e funções, com isso, podem melhorar a interação solo-microrganismos-plantas (GHOSH; GANGOPADHYAY, 2019; CHEN et al., 2019; RANDALL et al., 2019). As bactérias e os fungos representam os principais grupos de microrganismos presentes no solo, atuando diretamente em diversos processos deste ambiente, tais como decomposição e mineralização de compostos orgânicos e minerais (intemperismo), nos ciclos biogeoquímicos, na biorremediação de poluentes, na decomposição de xenobióticos e moléculas orgânicas, entre outros (SCHULZE et al., 2019; FINLAY et al., 2020).

A composição da comunidade do microbioma associado a rizosfera pode ser afetada por diversos fatores, desde condições físicas e químicas do solo, estádios de desenvolvimento e genótipos das plantas (LUNDBERG et al., 2012; MARQUES et al., 2014). Além disso, outros fatores como o tipo de preparo do solo e sistemas de cultivo de culturas (pousio, monocultivo, rotação de culturas, sistemas consorciados e modelos de sistema integração lavoura-pecuária-floresta) exercem influência sobre a diversidade da comunidade microbiana do solo (ANDREOTE; CARDOSO, 2016; PILLON et al., 2020).

No trabalho de Qiao et al. (2017) com diferentes tipos de solos, estádios fenológicos e genótipos de algodão, foi evidenciado que em solos com cultivo de algodão a longo prazo parte dos grupos microbianos do solo desaparecem gradualmente ou permanecem em um nível baixo, enquanto outra parte inicialmente em baixo nível é promovido pelo cultivo do algodão, com isso, a abundância destes aumentam. Isso sugere que o cultivo prolongado de plantas reduz a diversidade e aumenta abundância de determinados grupos microbianos, conforme também relatado por Moreira e Siqueira (2006).

Santoyo (2022) relata que a dinâmica populacional da rizosfera sob diferentes gradientes de oxigênio (O_2), pH e exsudatos radiculares, na presença de determinadas espécies microbianas, podem modular (ou ser moduladas) e influenciar o crescimento de plantas. Isso

pode promover o desenvolvimento de uma comunidade microbiana estimulada pela cultura do algodão e que contribui para seu crescimento e desenvolvimento do mesmo. Ao retratar o algodoeiro, este exibe forte simbiose mutualística com fungos micorrízicos arbusculares (SALGADO et al., 2017; NUNES et al., 2019), que promovem crescimento das plantas, principalmente por meio da maximização do uso de nutrientes do solo (MAI et al. 2018). Portanto, áreas com uma densidade e diversidade baixa deste fungo podem comprometer o desenvolvimento do algodão e exigindo assim maior entrada de fertilizante.

2.3 Fungos micorrízicos arbusculares

Aproximadamente mais de 80% das espécies vegetais vivem em simbiose mutualística com fungos micorrízicos arbusculares (FMA), grupo filogeneticamente uniforme do filo Glomeromycetes, caracterizado por uma baixa especificidade de hospedeiro (BALAMI et al., 2020). Esta associação denominada micorriza arbuscular, dentre os sete tipos existentes de micorrizícas (arbuscular, ectomicorriza, ectendomicorriza, arbutoide, monotropoide, ericoide e orquidoide), representam a simbiose mais abundante nos ecossistemas terrestres e de grande importância entre microrganismos e plantas (MOREIRA; SIQUEIRA, 2006).

Os FMA são microrganismos biotróficos obrigatórios, ou seja, cujo crescimento e desenvolvimento só ocorre obrigatoriamente em associação com as raízes de um hospedeiro vegetal metabolicamente ativo (MOREIRA; SIQUEIRA, 2006; MOHAN et al., 2014). A associação é caracterizada pela penetração das hifas inter e intracelulares ao colonizar o apoplasto e células corticais das raízes, formando arbúsculos, isso sem promover alterações morfológicas macroscópicas (MOREIRA; SIQUEIRA, 2006; STÜRMER; SIQUEIRA, 2013). Os arbúsculos são estruturas formadas pela ramificação das hifas, que por sua vez, conformam aspecto de uma pequena árvore nas células corticais e, são estas estruturas que garantem ao fungo acesso ao suprimento de carbono da planta (PARNISKE, 2008).

A associação das plantas com FMA promove aumento da absorção de água e nutrientes do solo, principalmente nutrientes imóveis e/ou parcialmente móveis (P, Cu e Zn) (SMITH; READ, 2008; FRESCHET et al., 2020; ALARCON et al., 2019), tolerância ao estresse hídrico, formação e estabilidade dos agregados do solo pela produção de micélios fúngicos e glicoproteínas (glomalina) (BARBOSA et al., 2019; FRESCHET et al., 2021), proteção da matéria orgânica e aumento do estoque de carbono orgânico (HOSSAIN, 2021), além disso, interação positiva com outros microrganismos benéficos do solo (MOREIRA; SIQUEIRA, 2006).

A comunidade de FMA é facilmente alterada conforme o manejo agrícola realizado, bem como, o tipo de cobertura vegetal, espécie em associação, fatores abióticos (temperatura, precipitação etc.). Outro grande exemplo de alteração é o revolvimento/movimentação do solo e, consequentemente a quebra do micélio do fungo proporciona a seleção de espécies que produzem menos micélio e maior quantidade de esporos, com isso, contribui para redução da diversidade de espécies de FMA (BALOTA et al., 2014).

Os FMA proporcionam efeitos positivos no crescimento do algodão, principalmente por meio da nutrição, todavia é válido ressaltar que a comunidade de FMA é variável de acordo com o sistema inserido (OEHL et al., 2009; PERREIRA et al., 2014). No estudo de Nunes et al. (2019) em bioma de Cerrado, em áreas produtoras de algodão branco convencional há mais de 10 anos, foram identificadas algumas espécies nativas de FMA pertencentes ao gênero *Acaulospora*, *Ambiospora*, *Claroideoglomus*, *Paraglomus*, *Dentisculada*, *Gigaspora*, *Glomus*, *Scutellospora* e *Rizoglomus*. Ainda sobre o mesmo estudo, todavia em áreas com apenas dois anos com o cultivo do algodão (histórico de sistema soja/milho em rotação), foram identificadas espécies de FMA do gênero *Acaulospora*, *Ambiospora*, *Claroideoglomus*, *Entrophospora*, *Glomus* e *Gigaspora*.

De acordo com o trabalho de Salgado et al. (2017) que trabalharam com a inoculação de cinco espécies de FMA na cultura do algodão, as espécies com melhor desempenho quanto ao aumento de matéria seca da parte aérea e acúmulo de nutrientes (Zn e P) nas plantas de algodão, foram *Acaulospora scrobiculata*, *Claroideoglomus etunicatum*, *Gigaspora margarita*, bem como a inoculação combinada.

Com base na literatura há uma modificação/alteração de grupos de FMA de acordo com o tempo de cultivo e sistema que o algodoeiro está inserido, além disso, certos grupos apresentam associação mais positiva à planta, principalmente quanto a absorção de nutrientes. No caso da problemática que motivou o presente estudo, o algodão substituindo o milho no sistema soja/milho pode estar iniciando um processo de recrutamento de microrganismos do solo, em especial FMA, e por esse motivo o seu crescimento, desenvolvimento e rendimento ficam aquém do esperado nos primeiros anos de cultivo.

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SEGUNDA PARTE

ARTIGO

Arbuscular mycorrhizal association is induced by long-term cotton cultivation and enhances P uptake and initial growth of cotton plants by legacy P exploration in soil

Versão preliminar – Normas do Journal of Soil Science and Plant Nutrition

Arbuscular mycorrhizal association is induced by long-term cotton cultivation and enhances P uptake and initial growth of cotton plants by legacy P exploration in soil

ABSTRACT

Purpose: production systems can affect soil properties, such as soil fertility and microbiological community and activity, favoring plant growth and crop yield. This study aimed to investigate the effect of successive cultivation systems on the chemical, biochemical, and biological properties of a tropical Oxisol and their relationship with cotton's initial growth and nutritional status. **Methods:** soil samples were collected in areas with different soybean/maize/cotton backgrounds (T0 - consolidated cultivation of soybean/maize; T1 - first year of cultivation in soybean/cotton; T2 - second year of soybean/cotton; T3 - third year of soybean/cotton; T4 - fourth year of soybean/cotton; T10 - tenth year of soybean/cotton). First, we evaluated the effect of T0 - T4 cultivation systems on soil properties and cotton initial growth (time cultivation experiment). Then, we evaluated the effect of dilution and autoclaving in T10 samples on soil properties and cotton growth (soil dilution/autoclaving experiment). Both experiments were carried out under greenhouse conditions. **Results:** in the time cultivation experiment, we observed that longer cultivation time increased P availability and P legacy index in soil and favored arbuscular mycorrhizal colonization in cotton roots and P uptake by cotton plants. Similarly, we observed in the soil dilution/autoclaving experiment that the sterilization limited the mycorrhizal colonization and induced P deficiency, even with P available above the critical limit in soil. **Conclusions:** the results indicated that the successive cultivation of cotton for several years (long-term) selected the mycorrhizal community, inducing higher colonization over the years, which is essential for P legacy utilization and cotton growth and production.

Key words: *Gossypium hirsutum* L. subsp. latifolium; arbuscular mycorrhizal fungi; legacy P index; degree of P saturation.

1 INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is a globally widespread crop cultivated on a large scale in many countries, such as China, India, the United States, and Brazil (Abrapa 2024). Cotton production is mainly destined for textile, paper, and cellulose industries, among other products. In addition, cotton seeds can be used in animal feed, oil, and biodiesel production (Stonex

2022). Although it presents highly weathered and low natural fertility tropical soils, the Cerrado region is the major cotton producer in Brazil, where farmers have invested in the soybean/cotton system, with cotton replacing maize in the soybean/maize system (Alves et al. 2018). However, cotton farmers have reported that the growth and productivity of cotton are impaired in the first harvests of soybean/cotton system, with a reduction of around 2000 kg ha^{-1} of seed cotton compared to established soybean/cotton systems, even under adequate soil chemical and physical conditions (Santos et al. 2023).

Many factors, including soil microbiological activity, can affect plant growth and development (Volpiano et al. 2022). Microorganisms participate in several biological processes, such as decomposition, nutrient mineralization and solubilization, nutrient transformation, and soil structuring (Moreira and Siqueira 2006; Sahu et al. 2018). In addition, root mutualistic symbioses with arbuscular mycorrhizal fungi (AMFs) can improve plant's access to nutrients and water due to the increased soil volume explored (Khaliq et al. 2022). Previous studies reported that cotton has a strong symbiosis with AMFs, favoring crop development mainly by increasing water and phosphorus (P) uptake (Wang et al. 2017). However, microbiological activity can be affected by plant species and soil management, modulating the structure of the microbial community associated with the rhizosphere (Moreira and Siqueira 2006; Schmidt et al. 2019).

There are no studies on the effect of cultivation systems on the microbiological activity of the soil and its relationship with nutrient availability, which can help elucidate the difference in cotton productivity between the first harvests and consolidated systems. Thus, the objective of the present study was to evaluate the effect of successive cultivation systems with cotton, soybean, and maize on nutrient availability, biochemical and microbiological properties of the soil, initial growth, and nutritional status of cotton plants in a greenhouse. Our main results demonstrate that the soil microbial community in soy/cotton consolidated systems favors the P uptake and initial growth of cotton plants, mainly through the increase in mycorrhizal association in roots, indicating that successive cotton cultivation selects fungi species able to mycorrhizal association, which is crucial for P legacy utilization by cotton plants.

2 MATERIAL AND METHODS

2.1 Soil experiments

Two soil experiments were carried out to investigate the relation between cultivation systems histories, chemical and microbiological soil properties, and growth and nutritional status of cotton seedlings, identified as cultivation time experiment (1) and soil dilution/autoclaving experiment (2).

2.1.1. Soil sampling and characterization

The soil samples used in the soil experiments were collected at Bigolim and Tucunaré farms in Sapezal, State of Mato Grosso, Brazil. According to the Köppen-Geiger classification, the region's climate is Aw, characterized by dry winters and humid summers, with average annual temperature and precipitation of 26 ºC and 1.750 mm, respectively. Soil samples were collected in the surface layer (0-15 cm depth). For the cultivation time experiment, soil samples were collected at Bigolim farm with a sample area divided according to the different succession histories of soybean/maize and soybean/cotton crops (Fig. 1). Three composite samples were collected in each area. Each sample was composed of five simple samples collected 20 m apart (Fig. 1). The soil was classified as Oxisol with a clayey texture (394 g kg^{-1} of clay, 158 g kg^{-1} of silt, and 448 g kg^{-1} of sand). For the soil dilution/autoclaving experiment, samples were collected at Tucunaré farm (latitude 12°59'22" S, longitude 58°45'22" W and 370 m altitude) in an area with ten years of soybean/cotton system. The soil was also classified as Oxisol with a clayey texture (405 g kg^{-1} of clay, 155 g kg^{-1} of silt, and 440 g kg^{-1} of sand). Chemical, biological, and biochemical properties of soil samples are presented in Tables S1 and S2.

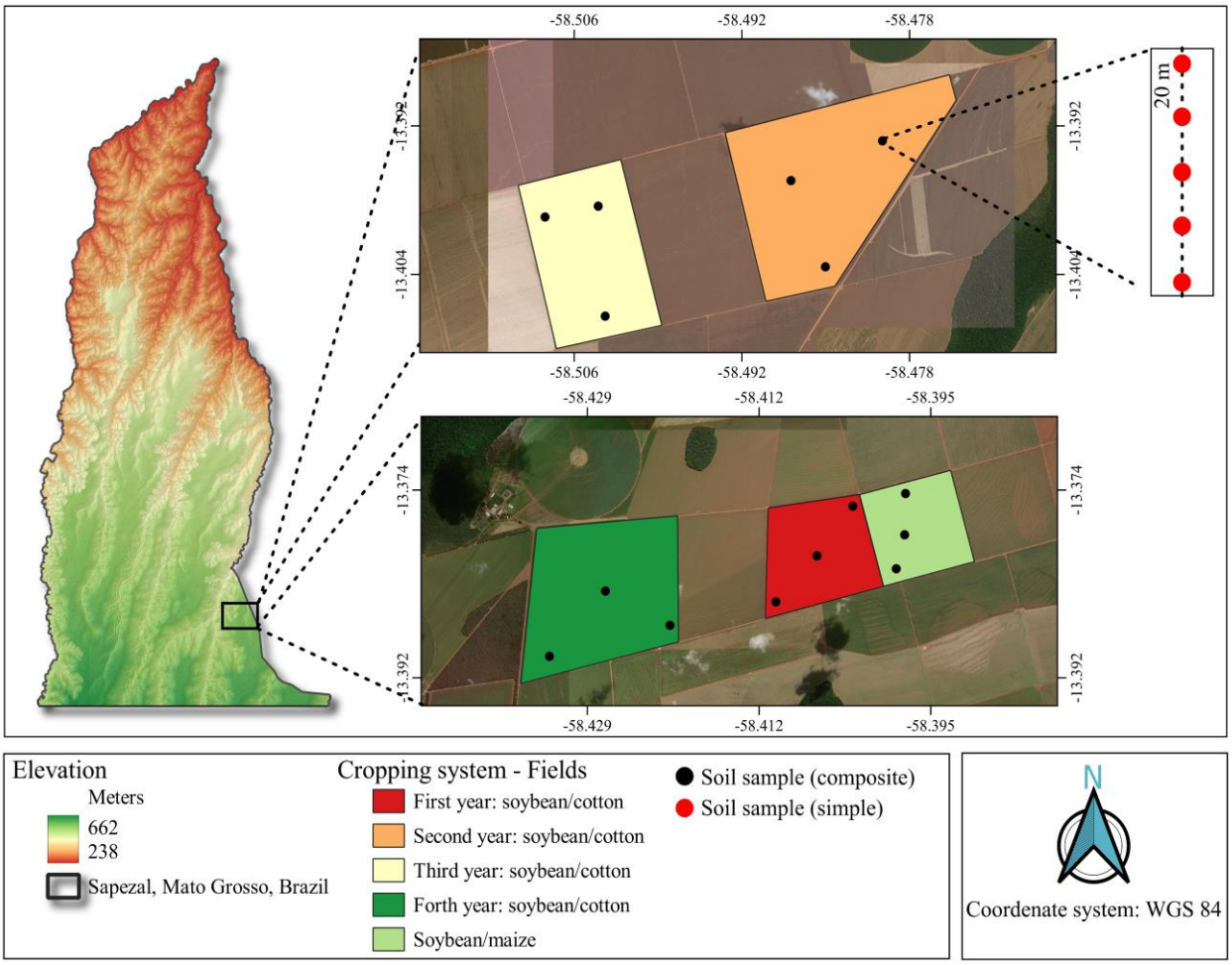

Fig. 1 Soil sampling areas for cultivation time experiment

2.1.2. Experimental designs

The objective of the cultivation time experiment was to evaluate the effect of soil from different successive soybean/maize/cotton cultivation histories on nutrient availability, soil biochemical and microbiological properties, initial growth, and nutritional status of cotton plants. The cultivation time experiment was carried out in a completely randomized design with five treatments and three replications. Each experimental unit consisted of one pot with two plants. The treatments consisted of different production system histories: T0 - consolidated cultivation with soybean/maize system; T1 - first year of soybean/cotton system cultivation; T2 - second year of soybean/cotton system; T3 - third year of the soybean/cotton system, and T4 - fourth year of the soybean/cotton system. The treatments were defined according to the different cultivation periods occurring in the State of Mato Grosso, Brazil, where the introduction of cotton replacing maize in the soy/maize system results in lower productivity of cotton plants around 2000 kg ha^{-1} of seed cotton. However, from the fourth year of the soybean/cotton system,

the cotton plant has productivity at adequate levels (> 6600 kg ha^{-1} of seed cotton), according to local cotton farmers.

The soil dilution/autoclaving experiment aimed to isolate and measure the effect of soil microorganisms from consolidated cultivation in a soybean/cotton system on nutrient availability, soil biochemical and microbiological properties, initial growth, and nutritional status of cotton plants. The soil dilution/autoclaving experiment was carried out in a 4 × 2 factorial scheme in a completely randomized design with three replications. Each experimental unit consisted of one pot with one plant. The factors correspond to four soil dilutions (25, 50, 75, and 100% of the soil to autoclaved sand) and two conditions of microbiological activity (AS – autoclaved soil and NAS – non-autoclaved soil). The soil used corresponds to ten years of successive soybean/cotton cultivation (T10), with productivity at desirable levels for cotton cultivation.

2.1.3. Experimental conditions and plant cultivation

Soil experiments were carried out under greenhouse conditions (11 h light/13 h dark, 19-30 ºC). Cotton seeds were sanitized before sowing (30 seconds in alcohol 70%, and two minutes in 2% sodium hypochlorite). Soil samples were sifted into using 2 mm mesh, and subsamples were collected for chemical, biological, and biochemical analysis. Soil moisture was maintained at 60% of field capacity.

In the cultivation time experiment, polypropylene pots (1.0 L capacity) were filled with soil samples according to the treatments. Then, five cotton seeds (*Gossypium hirsutum* - cv DP 1536 B2RF) were sown per pot. Seven days after sowing, thinning was performed to keep two plants in each pot. Plants were harvested 30 days after emergence.

In the soil dilution/autoclaving experiment, polypropylene pots (2.0 L capacity) were filled with soil/sand mixtures according to the treatments (Fig. S1). Soil samples and sand were autoclaved three times (120 ºC for 50 minutes with 24 hours rest). Then, five cotton seeds (*G. hirsutum* - cv FM 985 GLTP) were sown per pot. Seven days after sowing, thinning was performed to keep one plant in each pot. Plants were harvested 40 days after emergence.

2.2. Plant analysis

2.2.1. Growth evaluation and mycorrhization

At harvest times, plant height and stem diameter were measured, and shoots and roots were collected, rinsed with deionized water, and dried (55 ºC) for shoot (SDM) and root (RDM) dry matter determination. The total dry matter production (TDM) was obtained by the sum of SDM and RDM. Root samples (0.5 g fresh) were separated and cleaned for mycorrhization evaluation by staining method (Giovannetti and Mosse 1980).

2.2.2. Nutritional status

The SDM samples were milled for nutritional status evaluation. Samples (0.5 g) were subjected to nitro-perchloric digestion, and nutrient contents were determined by inductively coupled plasma atomic emission spectrometry (ICP-OES, Spectro, Blue, Germany). Nitrogen content was determined by the Kjeldahl method.

2.3. Soil analysis

2.3.1. Chemical analysis

Soil nutrient availability and acidity were evaluated according to Chitolina et al. (2009), and P fractionation was determined according to Gatiboni and Condron (2021). The P fractions evaluated were available P by Melich-3 (P-M3), inorganic P (Pi_{OH}), organic P (Po_{OH}), P extractable by HCl (P_{HCl}), occluded P (P_{ocl}), total P (P_{total}), and the P indexes calculated were degree of P saturation by Mehlich 3 (DPS_{M3}) and legacy P index (P_{legacy}).

2.3.2. Biological and biochemical analysis

Soil subsamples (rhizospheric soil) were stored under refrigeration at 4 ºC. Microbial biomass carbon (MBC) was evaluated by fumigation-extraction (soil dilution/autoclaving) (Vance, Brookes, and Jenkinson 1987) and irradiation (time cultivation) methods (Islam 1998). Basal soil respiration (RBS) was determined according to Alef (1995), and metabolic quotient (qCO_2) was calculated using MBC and RBS (Anderson and Domsch 1993). β-glucosidase, arylsulfatase, and acid phosphatase activities were measured by colorimetric methods

(Tabatabai and Bremner 1970; Eivazi and Tabatabai 1977). Hydrolysis of fluorescein diacetate (FDA) was measured according to Dick, Breakwell, and Turco (1996).

2.4 Statistical analysis

After checking normality (Shapiro-Wilk test) and homogeneity of variance (Bartlet test) (R Studio), all data were subjected to analysis of variance, and the means were compared by the Tukey test ($p \leq 0.05$), using the SISVAR software (Ferreira 2019). Pearson correlation analysis was performed using the R language using the Rstudio software.

3 RESULTS

3.1. Cultivation time experiment

3.1.1. Plant growth and mycorrhization

Plant growth and mycorrhization variables are presented in Fig. 2 and S2. The treatments did not affect plant growth. However, they influenced mycorrhization. The treatment with the longest time in soy/cotton succession (T4) increased the mycorrhization by 2-3 folds compared to treatments without/recent cotton cultivation (T0-T2) (Fig. 2e).

3.1.2. Nutritional status

Nutrient concentrations and accumulations are presented in Fig. 2, S3, and S4. P was the nutrient most influenced by treatments, showing effects in concentration and accumulation, while other nutrients were little affected, except S (Fig. 2e-g and Fig. S3). The T4 treatment increased the P concentration and accumulation by 38 and 48% compared to other treatments, respectively, promoting foliar P levels above critical limits, while it was below in the other treatments, indicating P deficiency. The other nutrients were found in adequate concentrations regardless of treatment (Fig.S3 and Fig.S4). P was the nutrient better correlated with AMF and SDM production, presenting positive correlations for P concentration and accumulation (Table S4).

3.1.3. Soil biological and biochemical properties

Soil biological and biochemical properties are shown in Table S1. The treatments did not affect markedly these properties, being in adequate conditions for soil health (Mendes et al. 2018).

3.1.4. Soil fertility and P fractions

Soil fertility properties and P fractionation are shown in Fig. 3 and Table S1. The treatments affected most P fractions, while they did not influence markedly the other properties, being all in adequate conditions for cotton cultivation (Bélot and Vilela 2020). In general, time cultivation increased the P fractions, with T3 and T4 treatments increasing available P extracted by Mehlich-3 solution (P-M3), P legacy (P-legacy), and degree of P saturation (DPS) indexes by 70, 36, and 63% compared to T0-T2 treatments, respectively (Fig. 3).

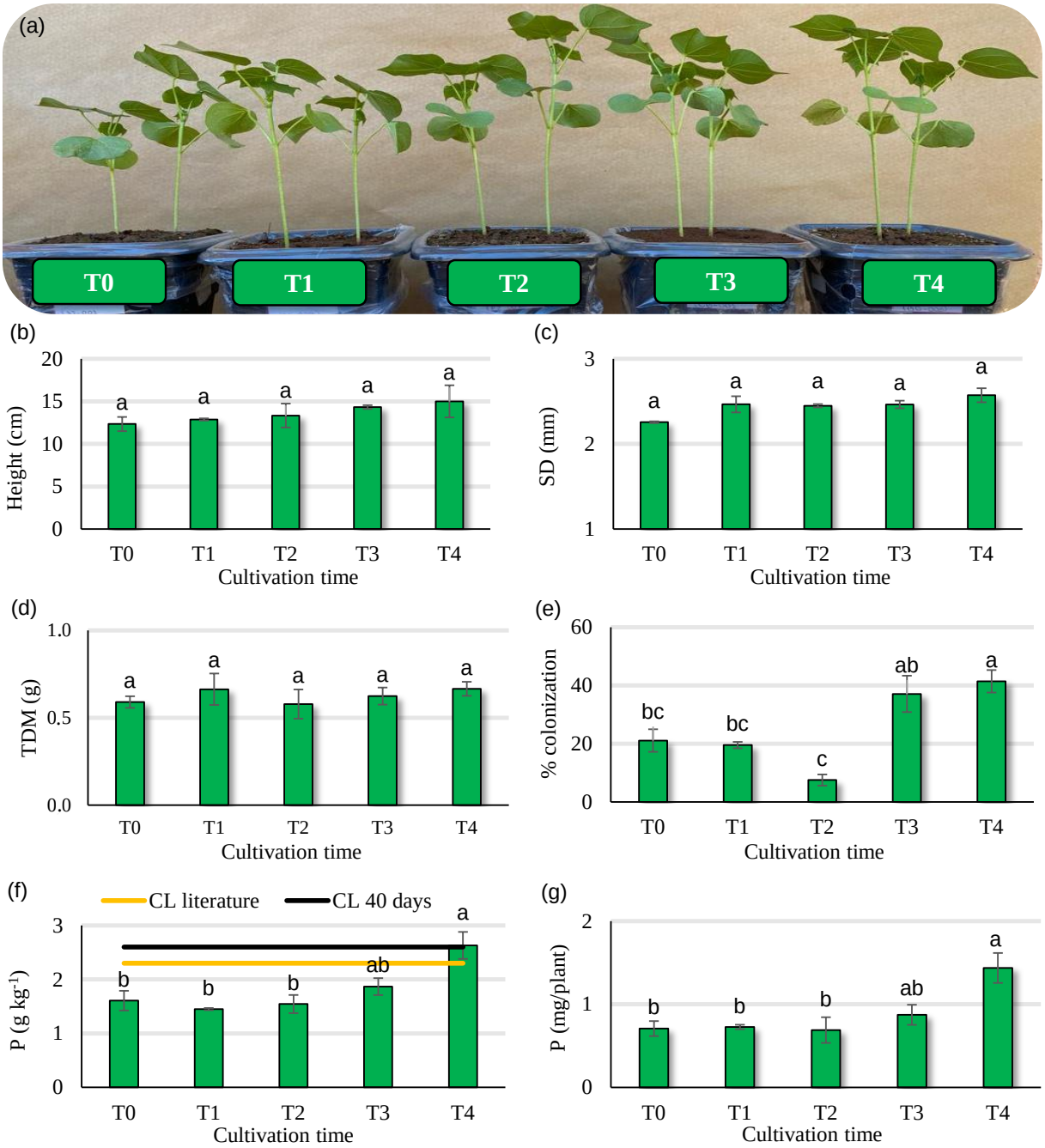

Fig. 2 Growth, mycorrhizal colonization, and P status in leaves of cotton plants grown in soils with different cultivation histories (cultivation time experiment). Plants at harvest time (a). Plant height (b). Stem diameter (c). Total dry matter (TDM, d). Mycorrhizal colonization of roots by arbuscular fungi (e). P concentration (f) and accumulation (g) in leaves. T0: soybean/maize cultivation; T1: soybean/cotton cultivation (first year); T2: soybean/cotton cultivation (second year); T3: soybean/cotton cultivation (third year); and T4: soybean/cotton cultivation (fourth year). CL 40 days: Critical level obtained in the hydroponic experiment (Fig. S10). CL literature: Critical level obtained in the literature for cotton (Kurihara et al. 2014). Columns with the same letters do not differ by Tukey test ($p > 0.05$)

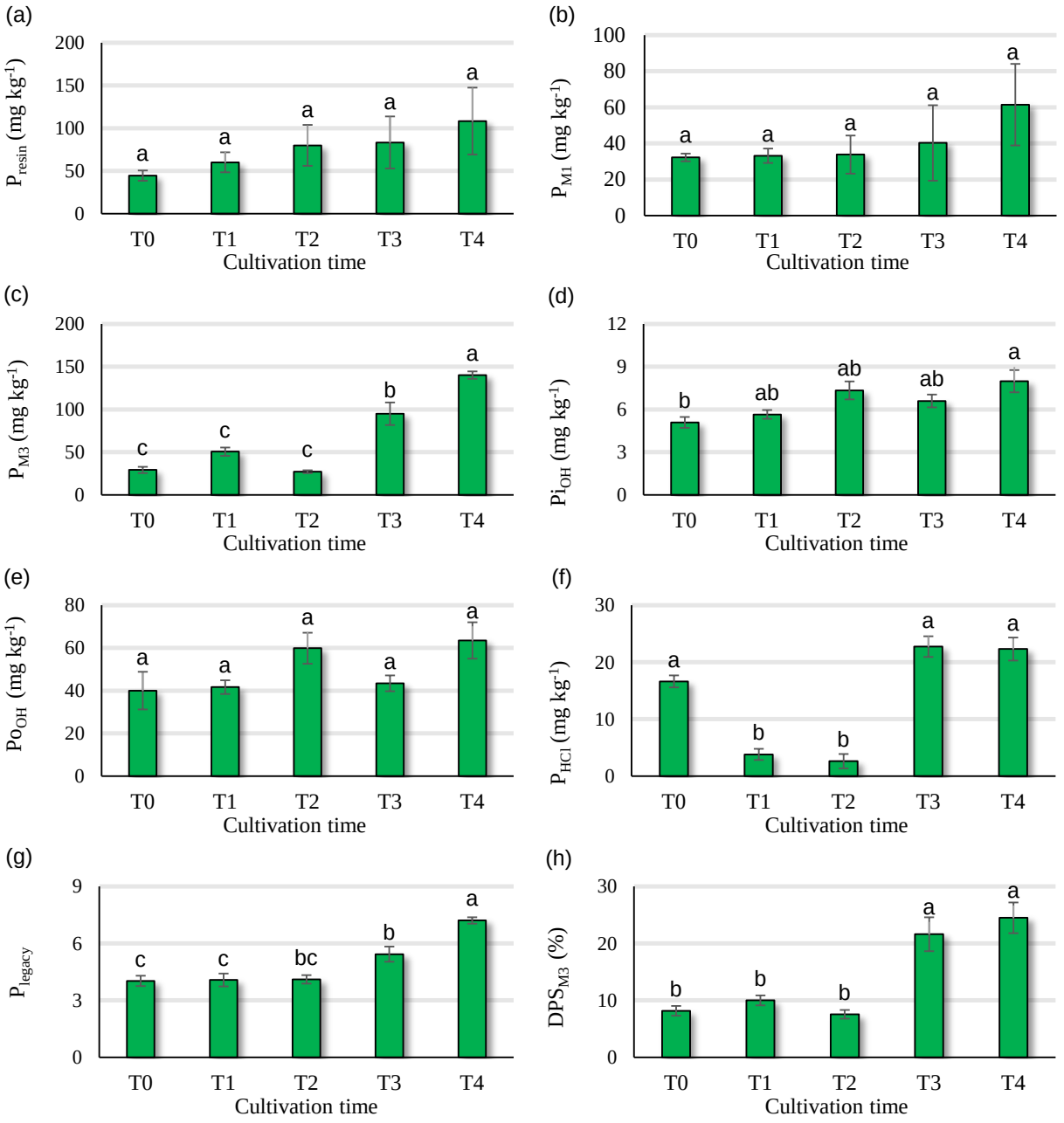

Fig. 3 Phosphorus fractions and availability indexes in soils with different cultivation histories (cultivation time experiment). P available extracted with resin (a), Mehlich-1 (b), and Mehlich-3 (c). Inorganic (d), organic P (e), and calcium-bounded P (P_{HCl}, f) fractions. P legacy (g) and degree of P saturation by Mehlich-3 (DPS_{M3}, h) indexes. T0: soybean/maize cultivation; T1: soybean/cotton cultivation (first year); T2: soybean/cotton cultivation (second year); T3: soybean/cotton cultivation (third year); and T4: soybean/cotton cultivation (fourth year). Columns with the same letters do not differ by Tukey test ($p > 0.05$)

3.2. Soil dilution/autoclaving experiment

3.2.1. Plant growth and mycorrhization

Plant growth and mycorrhization variables are presented in Fig. 4 and Fig. S6. The treatments affected all variables, improving plant growth and mycorrhization in NAS with higher soil proportions. The 100% NAS increased height (28%), stem diameter (25%), and total dry matter (49%) compared to 100% AS (Fig. 4b-d). Similarly, mycorrhization was markedly higher in NAS, independently of dilution, with 100% NAS promoting mycorrhization 29 folds higher than 100% AS (Fig. 4e).

3.2.2. Nutritional status of plants

Nutrient concentrations and accumulations are presented in Fig. 4, S7, and S8. The treatments affected all nutrients evaluated; however, they were above the foliar critical levels regardless of the treatment, except for P (Kurihara et al. 2014). The treatments with NAS presented P foliar markedly higher than AS, with 100% NAS increasing P concentration and accumulation by 63 and 80%, respectively, compared to 100% AS (Fig. 4f-g). In addition, the foliar P was above the critical limit only in NAS treatments, with it being below in the AS, even at 100% AS, indicating P deficiency. P in leaves presented stronger correlations with TDM and mycorrhization compared to other nutrients (Table S4), indicating that the treatment's effect on plant growth is due to mycorrhizal association and consequent increase in P uptake.

3.2.4. Soil biological and biochemical properties

Soil biological and biochemical properties are shown in Table S5. The treatments affected all properties evaluated, with autoclavation efficiency indicated mainly by MBC, arylsulfatase, β-glucosidase, and acid phosphatase reduced values.

3.2.5. Soil fertility and P fractions

Soil fertility properties and P fractionation are shown in Fig. 5 and Table S3. The treatments affected most properties, mainly due to soil proportions, while the autoclavation did not affect the properties, except for P available by resin (P-resin) and P-M3 at 100% proportion.

The 100% NAS increased P-resin and P-M3 by 21 and 41% compared to 100% AS, respectively (Fig. 5).

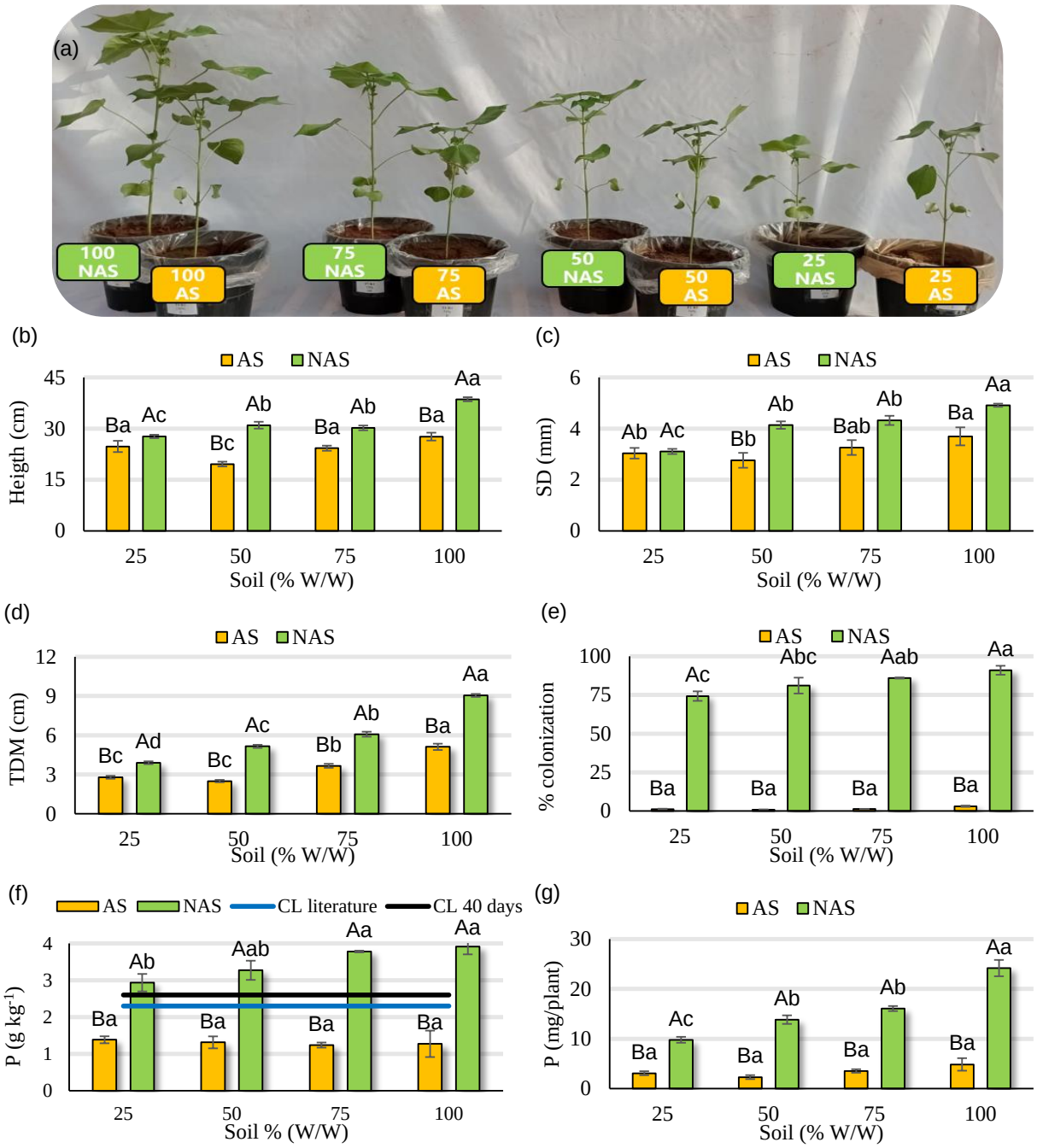

Fig. 4 Growth, mycorrhizal colonization, and P status in leaves of cotton plants grown in different soil dilutions and microbiological activity conditions (soil dilution/autoclaving experiment). Plants at harvest time (a). Plant height (b). Stem diameter (c). Total dry matter (TDM, d). Mycorrhizal colonization of roots by arbuscular fungi (e). P concentration (f) and accumulation (g) in leaves. AS: autoclaved soil. NAS: non-autoclaved soil. 25, 50, 75, and 100: soil/sand ratio % (W/W). CL 40 days: Critical level obtained in the hydroponic experiment (Fig. S10). CL literature: Critical level obtained in the literature for cotton (Kurihara et al. 2014). Uppercase letters compare microbiological activity conditions (AS and NAS) in each dilution, and lowercase letters compare dilutions in each microbiological condition by Tukey test ($p \leq 0.05$)

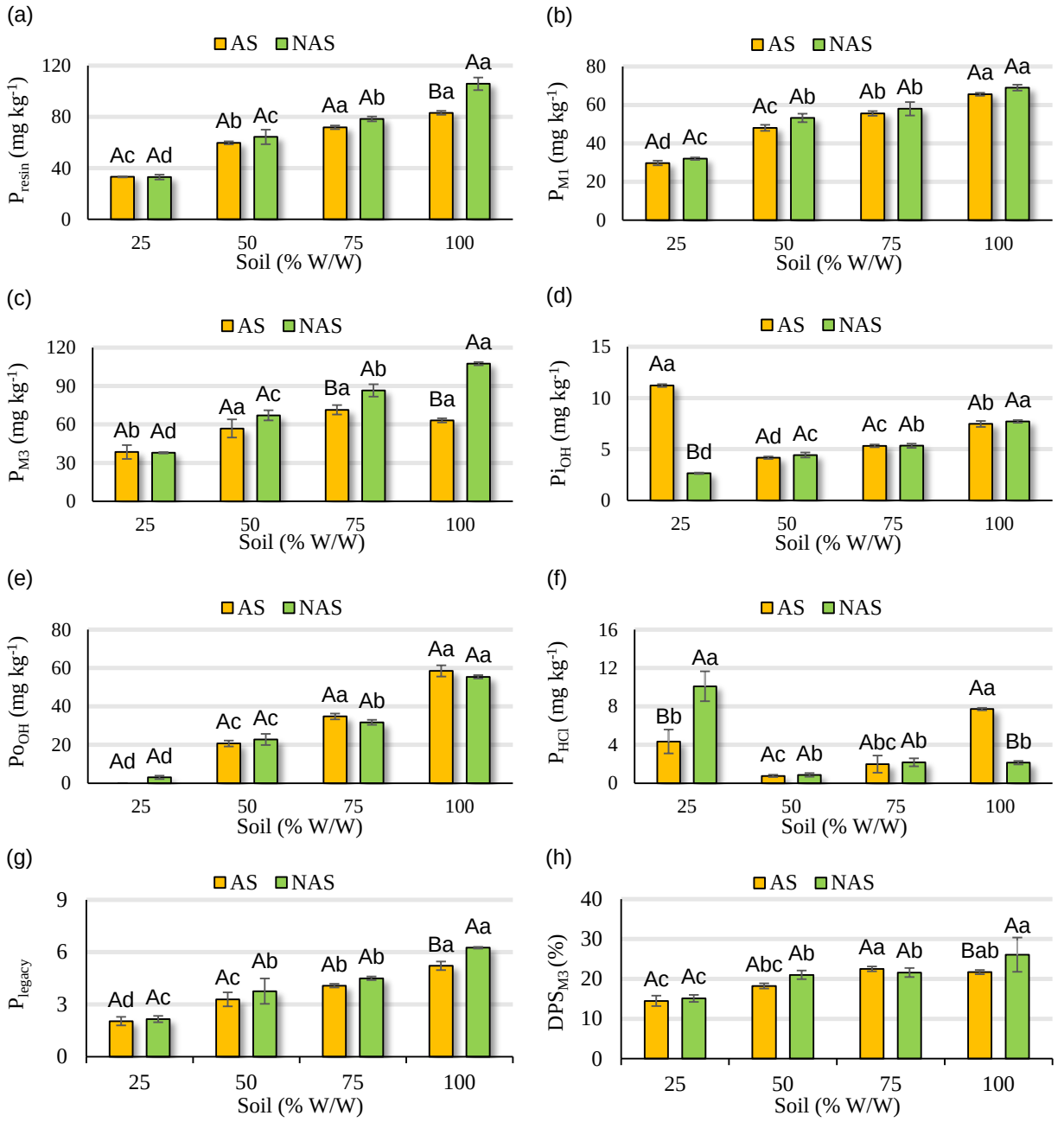

Fig. 5 Phosphorus fractions and availability indexes in soils with different dilutions (soil/autoclaved sand) and microbiological activity conditions (soil dilution/autoclaving experiment). P available extracted with resin (a), Mehlich-1 (b), and Mehlich-3 (c). Inorganic (d), organic P (e), and calcium-bounded P (P_{HCl}, f) fractions. P legacy (g) and degree of P saturation by Mehlich-3 (DPS_{M3}, h) indexes. AS: Autoclaved soil. NAS: non-autoclaved soil. 25, 50, 75, and 100: soil/sand ratio % (W/W). Uppercase letters compare microbiological activity conditions (AS and NAS) in each dilution, and lowercase letters compare dilutions in each microbiological condition by Tukey test ($p \leq 0.05$)

4 DISCUSSION

The effect of cultivation time on cotton growth is mainly related to increases in P availability and uptake and mycorrhizal colonization by arbuscular fungi in the roots at both cultivation time and soil dilution experiments. Despite the lack of difference in plant growth in the cultivation time experiment (Fig. 2b-d), we observed a significant increase in the concentration and accumulation of P and colonization according to increasing cultivation time with the soybean/cotton system (Fig. 2e-g). In the dilution experiment, the preservation of microbiological activity in long-term soybean/cotton cultivation soil also showed an increase in these variables. P is a vital nutrient for photosynthesis, respiration, energy transfer, cell division, and plant growth and development (Barra et al. 2019). In the cultivation time experiment, P showed strong correlations with plant growth, and only treatment with the longer cotton cultivation (T4) presented a concentration above the critical level.

The cultivation time increased the available P in the soil (P-M3) (Fig. 3c), while the other nutrients were little affected (Table S1). This effect is probably related to residual P, which consists of a positive balance between P input through fertilization and P output, mainly by the exportation of plant material harvested (Sattari et al. 2012). The soils of the Cerrado have a high content of total P; however, a small part is readily available to plants due to the predominance of highly weathered soils (Santos et al. 2018) with a strong P adsorption on low-activity clay minerals and Fe and Al oxides (Roy et al. 2016). On the other hand, Rodrigues et al. (2016) demonstrated that the successive cultivation of soybean/cotton increases labile P and moderately labile P over time due to annual P additions that saturate P adsorption sites, reducing P binding energy and leaving more for plants (Barrow, Barman, and Debnath 2018). Thus, the longer cultivation time contributed to higher P availability and uptake, as indicated by the correlations between concentration and accumulation of P in plants and fractions of available P in the soil (Table S4).

However, the results obtained in the dilution/autoclaving experiment demonstrate that microorganisms are essential for taking advantage of the higher P availability since in 100% AS the P content was almost three times lower than in 100% NAS (Fig. 4f). Thus, the more significant mycorrhizal colonization according to the longer cultivation time with soybean/cotton system (Fig. 4e) may be partially responsible for the better growth of cotton plants and consequently, productivity as observed by cotton farmers in already consolidated cultivation areas. It is well known that AMFs favor P uptake mainly by increasing the volume of explored soil. Mycorrhization favors cotton growth and P absorption, especially in the early

stages of plant development (Eskandari et al. 2017). However, root colonization and P accumulation in cotton can vary according to the AMF species (Salgado et al. 2017).

Compounds exudated by plants in the rhizosphere can favor AMFs and P-solubilizing microorganisms, which are strategic for exploring the legacy of P in the soil. Since plants comprise one of the main factors that modulate the soil microbial community through the exudation of compounds, it has been reported that cotton cultivation increases some microbial groups. In contrast, others are reduced (Qiao et al. 2017), modifying the structural diversity of bacterial communities (Wei and Yu 2018). Thus, the transition from soybean/maize to soybean/cotton can generate recruitment of benefic microorganisms stimulated by cotton, mainly AMF, resulting in the greater colonization observed in plants grown in soil with longer cultivation time (Fig. 2e). Thus, this study demonstrates the importance of the microbiota in more prolonged cotton cultivation at production systems, even in areas of high soil fertility, suggesting that strategic biological management can improve cotton yield. Furthermore, future research is necessary to identify microorganisms recruited in established soybean/cotton systems, mainly mycorrhizal fungi species.

5 CONCLUSIONS

The prolonged cultivation with the soybean/cotton system stimulates the arbuscular mycorrhizal fungi community in soil, favoring the mycorrhizal colonization of cotton roots. Mycorrhizal colonization is essential for P legacy exploration, improving P uptake and cotton growth. Future studies about species identification and inoculation of these microorganisms and the effect of cotton root exudation in selecting these beneficial microorganisms are still necessary.

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Supplementary material

Table S1. Chemical and biological characterization of soil samples with different cultivation histories used in cultivation time experiment (T0-T4)

	T0	T1	T2	T3	T4
pH	5.80 ± 0.30	5.50 ± 0.20	5.40 ± 0.20	5.50 ± 0.20	5.60 ± 0.10
SOM	4.22 ± 0.25	5.14 ± 0.33	4.54 ± 0.13	4.10 ± 0.16	3.57 ± 0.35
K	121.98 ± 17.43	101.72 ± 7.51	139.66 ± 16.30	107.84 ± 3.00	122.47 ± 1.83
P-M1	32.20 ± 3.70	33.10 ± 6.9	33.80 ± 9.90	39.8 ± 4.80	40.20 ± 11.10
P-resin	44.50 ± 10.40	60.10 ± 7.80	58.20 ± 17.40	83.30 ± 13.10	69.80 ± 10.80
Ca	4.27 ± 0.52	4.38 ± 0.20	3.88 ± 0.44	3.56 ± 0.28	3.96 ± 0.39
Mg	1.62 ± 0.27	1.28 ± 0.11	1.16 ± 0.37	1.24 ± 0.16	1.07 ± 0.24
S	6.17 ± 0.44	20.13 ± 4.31	24.13 ± 2.68	20.30 ± 3.12	16.77 ± 0.58
Al	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
H + Al	2.50 ± 0.31	3.43 ± 0.28	4.17 ± 0.34	3.27 ± 0.52	3.57 ± 0.49
P-rem	26.60 ± 1.53	29.40 ± 0.32	24.43 ± 1.55	30.73 ± 5.04	34.43 ± 3.33
Zn	5.10 ± 0.36	7.50 ± 0.44	4.67 ± 0.98	5.67 ± 0.92	7.63 ± 0.96
Fe	26.20 ± 1.21	25.77 ± 1.33	26.73 ± 0.97	20.53 ± 2.06	23.77 ± 1.71
Mn	14.33 ± 2.23	16.17 ± 1.07	13.50 ± 2.48	12.47 ± 0.86	15.20 ± 1.08
B	0.26 ± 0.01	0.31 ± 0.01	0.28 ± 0.01	0.21 ± 0.02	0.25 ± 0.04
CEC	8.70 ± 0.53	9.35 ± 0.26	9.56 ± 0.51	8.35 ± 0.30	8.91 ± 0.28
V (%)	70.77 ± 4.72	63.32 ± 2.71	55.87 ± 5.53	61.05 ± 5.73	59.81 ± 6.11
m (%)	1.20 ± 0.61	1.09 ± 0.55	1.90 ± 0.25	1.96 ± 0.18	1.89 ± 0.23
	T0	T1	T2	T3	T4
MBC	221.79 ± 31.09	240.97 ± 43.66	238.78 ± 20.53	218.22 ± 17.60	222.08 ± 17.21
BSR	3.13 ± 0.37	2.18 ± 0.30	2.45 ± 0.36	2.78 ± 0.39	3.53 ± 0.38
qCO_2	14.70 ± 1.57	9.58 ± 0.89	10.89 ± 2.02	13.23 ± 1.05	16.52 ± 2.17
Arylsulfatase	381.00 ± 134.00	508.00 ± 117.00	472.00 ± 27.00	287.00 ± 4.90	301.00 ± 21.00
Acid phosphatase	1813.00 ± 255.00	2413.00 ± 257.00	2453.00 ± 135.00	1732.00 ± 115.00	1883.00 ± 178.00
β-glucosidase	649.00 ± 160.00	795.00 ± 122.00	758.00 ± 146.00	568.00 ± 68.00	607.00 ± 119.00
FDA	306.33 ± 16.17	352.12 ± 38.16	225.43 ± 24.05	305.65 ± 23.33	276.31 ± 23.12

T0: soybean/maize cultivation; T1: soybean/cotton cultivation (first year); T2: soybean/cotton cultivation (second year); T3: soybean/cotton cultivation (third year); T4: soybean/cotton cultivation (fourth year), pH in water (soil-to-solution ratio 1:2.5). SOM: soil organic matter (dag kg^{-1}). P-M1: P extracted by Mehlich-1 solution. P-resin: P extracted by ion exchange resin. H + Al: potential acidity estimated at pH 7.0. P-rem: remaining P (mg L^{-1}). CEC: cation exchange capacity (Ca + Mg + K + 'H + Al'). V (%): base saturation [(Ca + Mg + K) / (Ca + Mg + K + 'H + Al')] x 100. m (%): aluminum saturation [(Al) / (Al + Ca + Mg + K)] x 100. K, P-M1, P-resin, S, Zn, Fe, Mn, and B: mg dm^{-3}. Ca, Mg, Al, 'H + Al', and CEC: $cmol_c$ dm^{-3}. MBC: microbial biomass carbon (μg g^{-1} C). BSR: basal soil respiration (mg CO_2 g^{-1} h^{-1}). qCO_2: metabolic quotient (C-CO_2 μg^{-1} MBC h^{-1}). Arylsulfatase (μg PNF g^{-1} h^{-1}). Acid phosphatase (μg PNF g^{-1} h^{-1}). β-glucosidase (μg PNF g^{-1} h^{-1}). PNF: p-nitrophenol. FDA: hydrolysis of fluorescein diacetate (μg F g^{-1} day^{-1}). Average of three replications ± standard error

Table S2. Chemical and biological characterization of soil samples from a consolidated soybean/cotton system (≥ 10 years old -T10) used in the dilution/autoclaving experiment

pH	SOM	K	P-M1	P-resin	Ca	Mg	S	Al
6.6 ± 0.03	4.5 ± 0.3	47 ± 3	69 ± 1.54	106 ± 5	6 ± 0.2	2.4 ± 0.1	17 ± 2.1	0.12 ± 0.01
H + Al	P-rem	Zn	Fe	Mn	B	CEC	V (%)	m (%)
1.43 ± 0.03	34 ±1.7	11.2 ±0.5	79 ± 6	45 ± 2	0.21 ± 0.03	8.6 ± 0.3	86 ± 0.7	1.43 ± 0.04
MBC	BSR	qCO_2	Arylsulfatase	Acid phosphatase		β-glucosidase		FDA
751 ± 56	2.9 ± 0.5	3.95 ± 0.56	218 ± 29	205 ± 16		125 ± 4		53 ± 3

pH in water (soil-to-solution ratio 1:2.5). SOM: soil organic matter (dag kg^{-1}). P-M1: P extracted by Mehlich-1 solution. P-resin: P extracted by ion exchange resin. H + Al: potential acidity estimated at pH 7.0. P-rem: remaining P (mg L^{-1}). CEC: cation exchange capacity (Ca + Mg + K + 'H + Al'). V (%): base saturation [(Ca + Mg + K) / (Ca + Mg + K + 'H + Al')] x 100. m (%): aluminum saturation [(Al) / (Al + Ca + Mg + K)] x 100. K, P-M1, P-resin, S, Zn, Fe, Mn, and B: mg dm^{-3}. Ca, Mg, Al, 'H + Al', and CEC: $cmol_c$ dm^{-3}. MBC: microbial biomass carbon (µg g^{-1} C). BSR: basal soil respiration (mg CO_2 g^{-1} h^{-1}). qCO_2: metabolic quotient (C-CO_2 µg^{-1} MBC h^{-1}). Arylsulfatase (µg PNF g^{-1} h^{-1}). Acid phosphatase (µg PNF g^{-1} h^{-1}). β-glucosidase (µg PNF g^{-1} h^{-1}). PNF: p-nitrophenol. FDA: hydrolysis of fluorescein diacetate (µg F g^{-1} day^{-1}). Average of three replications ± standard error

Table S3. Chemical attributes in different proportions of soil/autoclaved sand (soil dilution/autoclaving experiment)

Dilution	**-------- pH --------**		**-------- SOM --------**		**-------- K --------**		**-------- P-M1 --------**		**-------- P-resin --------**		**-------- Ca --------**	
% (W/W)	AS	NAS	AS	NAS	AS	NAS	AS	NAS	AS	NAS	AS	NAS
25	6.50 ns	6.13 ns	1.36 Ad	1.14 Ac	18.48 Ac	23.90 Ac	29.77 Ad	32.01 Ac	33.1 Ac	33.0 Ad	2.22 Ad	2.37 Ac
50	6.67 ns	6.77 ns	2.31 Ac	2.80 Ab	38.67 Ab	33.63 Ab	48.06 Ac	53.21 Ab	59.7 Ab	64.3 Ac	3.94 Ac	4.03 Ab
75	6.77 ns	6.60 ns	3.29 Ab	3.82 Aa	47.18 Aab	39.94 Bab	55.57 Ab	57.98 Ab	71.8 Aa	78.4 Ab	4.93 Ab	4.49 Ab
100	6.63 ns	6.73 ns	4.42 Aa	4.50 Aa	50.60 Aa	47.30 Aa	65.54 Aa	68.99 Aa	83.2 Ba	105.8 Aa	5.87 Aa	5.95 Aa
D	2.00 ns		118.90**		52.60**		144.60**		152,40**		101,90**	
A	0.40 ns		2.90 ns		2.30 ns		6.80**		16.20**		0.04 ns	
D X A	0.72 ns		1.97 ns		2.80 ns		0.30 ns		5.60**		0.90 ns	
CV (%)	9.94		10.46		10.86		6.06		7.74		8.70	
Dilution	**-------- Mg --------**		**-------- S --------**		**-------- Al --------**		**-------- H + Al --------**		**-------- P-rem --------**		**-------- Zn --------**	
% (W/W)	AS	NAS	AS	NAS	AS	NAS	AS	NAS	AS	NAS	AS	NAS
25	0.96 Aa	0.95 Ac	5.77 Ad	6.20 Ab	0.09 Ab	0.08 Ac	0.72 Ac	0.75 Ab	53.54 Aa	43.80 Ba	3.64 Ac	4.00 Ad
50	1.72 Ac	1.66 Ab	10.31 Ac	9.23 Ab	0.13 Aa	0.09 Bbc	1.02 Ab	1.16 Aa	44.73 Ab	40.29 Aab	6.58 Ab	7.40 Ac
75	2.09 Ab	1.96 Ab	17.07 Ab	9.20 Bb	0.14 Aa	0.11 Bab	1.10 Ab	1.29 Aa	38.01 Abc	36.81 Aab	7.70 Bb	9.12 Ab
100	2.53 Aa	2.44 Aa	24.00 Aa	17.40 Ba	0.15 Aa	0.12 Ba	1.39 Aa	1.43 Aa	35.26 Ac	34.31 Ab	10.36 Aa	11.21 Aa
D	101.10**		69.70**		43.50**		31.90**		16.10**		178.17**	
A	1.30 ns		25.70**		42.90**		4.20 ns		7.20		15.64**	
D X A	0.10 ns		7.50**		2.70 ns		0.60 ns		1.80 ns		1.02 ns	
CV (%)	8.80		14.81		7.35		11.00		9.14		7.13	
Dilution	**-------- Fe --------**		**-------- Mn --------**		**-------- B --------**		**-------- CEC --------**		**-------- V --------**		**-------- m --------**	
% (W/W)	AS	NAS	AS	NAS	AS	NAS	AS	NAS	AS	NAS	AS	NAS
25	70.60 Aa	53.1 Ac	29.0 Ac	28.0 Ac	0.22 Aa	0.16 Ba	3,31 Ac	3,46 Ac	81,56 ns	81,98 ns	2,66 a	2,32 a
50	43.72 Bb	87.5 Aa	35.2 Ab	38.8 Ab	0.26 Aa	0.20 Ba	5.63 Ab	5.87 Ab	85.01 ns	83.10 ns	2.32 ab	1.66 b
75	38.19 Bb	57.5 Abc	38.7 Ab	38.0 Ab	0.24 Aa	0.20 Aa	8.24 Aa	6.83 Bb	85.80 ns	84.11 ns	1.75 bc	1.62 b
100	38.28 Bb	78.7 Aab	44.5 Aa	45.3 Aa	0.29 Aa	0.21 Ba	8.63 Aa	8.64 Aa	85.72 ns	85.60 ns	1.69 c	1.43 b
D	3.33**		47.90**		3.23 ns		105.27 **		1.93 ns		16.80 **	
A	26.27**		0.40 ns		18.13**		1.04 ns		0.21 ns		5.31**	
D X A	11.28**		0.40 ns		0.46 ns		3.13 ns		0.21 ns		1.11 ns	
CV (%)	12.57		6.42		15.51		8.58		3.56		12.88	

D: dilution; A: autoclaving. AS: autoclaved soil and NAS: non-autoclaved soil. 25, 50, 75, and 100: soil/sand ratio % (W/W). pH in water (soil-to-solution ratio 1:2.5). SOM: soil organic matter (dag kg^{-1}). P-M1: P extracted by Mehlich-1 solution. P-resin: P extracted by ion exchange resin. H + Al: potential acidity estimated at pH 7.0. P-rem: remaining P (mg L^{-1}). CEC: cation exchange capacity (Ca + Mg + K + 'H + Al'). V (%): base saturation [(Ca + Mg + K) / (Ca + Mg + K + 'H + Al')] x 100. m (%): aluminum saturation [(Al) / (Al + Ca + Mg + K)] x 100. K, P-M1, P-resin, S, Zn, Fe, Mn, and B: mg dm^{-3}. Ca, Mg, Al, 'H + Al', and CEC: $cmol_c$ dm^{-3}. Uppercase letters compare microbiological activity conditions (AS and NAS) in each dilution, and lowercase letters compare dilutions in each microbiological condition by Tukey test ($p \leq 0.05$). ** $p \leq 0.05$. ns: not significant

Table S4. Correlations between growth variables, arbuscular mycorrhizal colonization (AMF), concentration and accumulation of nutrients and soil P fractions in cotton plants grown in soils with different cultivation histories (cultivation time experiment) or dilution conditions and microbiological activity (dilution experiment soil/autoclaving)

	Nutrient concentration in leaves – Cultivation time experiment								
	P	K	Ca	Mg	S	Zn	Fe	Mn	B
SDM	0.50***	0.09^{ns}	0.04^{ns}	-0.24^{ns}	0.34^{ns}	-0.35^{ns}	-0.10^{ns}	-0.18^{ns}	-0.11^{ns}
RDM	0.17^{ns}	-0.28^{ns}	-0.22^{ns}	-0.49^{ns}	0.23^{ns}	-0.49^{ns}	-0.20^{ns}	-0.34^{ns}	-0.18^{ns}
TDM	0.48^{ns}	0.02^{ns}	0.00^{ns}	-0.31^{ns}	0.35^{ns}	-0.41^{ns}	-0.13^{ns}	-0.22^{ns}	-0.14^{ns}
AMF	0.66*	-0.16^{ns}	0.33^{ns}	0.07^{ns}	0.27^{ns}	0.27^{ns}	0.23^{ns}	0.17^{ns}	0.43^{ns}

	Nutrient accumulation in leaves – Cultivation time experiment								
	P	K	Ca	Mg	S	Zn	Mn	Fe	B
SDM	0.83**	0.86**	0.85**	0.77**	0.74**	0.43^{ns}	0.31^{ns}	0.72**	0.83**
RDM	0.33^{ns}	0.22^{ns}	0.27^{ns}	0.13^{ns}	0.39	-0.12^{ns}	-0.13^{ns}	0.25^{ns}	0.31^{ns}
TDM	0.81**	0.81**	0.81**	0.71**	0.74**	0.36^{ns}	0.25^{ns}	0.69**	0.80**
AMF	0.56*	-0.14^{ns}	0.12^{ns}	-0.03^{ns}	0.16^{ns}	0.22^{ns}	0.16	0.11^{ns}	0.21^{ns}

	Nutrient concentration in leaves – Soil dilution/autoclaving experiment									
	N	P	K	Ca	Mg	S	Zn	Fe	Mn	B
SDM	-0.46*	0.74**	-0.25^{ns}	0.63**	0.61**	0.39^{ns}	-0.30^{ns}	-0.39^{ns}	-0.62**	-0.52**
RDM	-0.28^{ns}	0.63**	-0.13^{ns}	0.61**	0.58**	0.60**	-0.13^{ns}	-0.43*	-0.47*	-0.27^{ns}
TDM	-0.41*	0.73**	-0.22^{ns}	0.65**	0.62**	0.48*	-0.24^{ns}	-0.42*	-0.59**	-0.45*
AMF	-0.84**	0.96**	-0.73*	0.80**	0.69**	0.01^{ns}	-0.63**	-0.18^{ns}	-0.95**	-0.55**

	Nutrient accumulation in leaves – Soil dilution/autoclaving experiment									
	N	P	K	Ca	Mg	S	Mn	Zn	Fe	B
SDM	0.34^{ns}	0.90**	0.72**	0.98**	0.98**	0.93**	-0.16^{ns}	0.59**	0.39^{ns}	0.70**
RDM	0.30^{ns}	0.83**	0.63**	0.87**	0.86**	0.92**	-0.19^{ns}	0.57**	0.14^{ns}	0.69**
TDM	0.34^{ns}	0.91**	0.71**	0.97**	0.98**	0.96**	-0.18^{ns}	0.60**	0.31^{ns}	0.72*
AMF	-0.29^{ns}	0.89**	0.14^{ns}	0.81**	0.78**	0.57**	-0.69**	0.07^{ns}	0.41*	0.31^{ns}

	Soil P fractions – Cultivation time experiment									
	P M1	P resin	P total	P M3	PiOH	PoOH	PHCl	P occluded	P legacy	DPS M3
P concentration	0.81**	0.76**	0.70**	0.76**	0.70**	0.55*	0.69**	0.43^{ns}	0.90**	0.69**
P accumulation	0.88**	0.82**	0.72**	0.76**	0.69**	0.52^{ns}	0.63*	0.45^{ns}	0.90**	0.90*

	Soil P fractions – Soil dilution/autoclaving experiment									
	P M1	P resin	P total	P M3	PiOH	PoOH	PHCl	P occluded	P legacy	DPS M3
P concentration	0.26^{ns}	0.34^{ns}	0.19^{ns}	0.55**	-0.22^{ns}	0.14^{ns}	-0.13^{ns}	0.17^{ns}	0.33^{ns}	0.37^{ns}
P accumulation	0.47*	0.56**	0.44*	0.73**	-0.06^{ns}	0.38^{ns}	-0.18	0.41*	0.57**	0.58**

SDM: shoot dry matter; RDM: root dry matter; TDM: total dry matter; *, ** and ***: $p \leq 0.05$, 0.01 and 0.07, respectively. ns: not significant. Phosphorus availability fractions and indices: available P extracted with resin (P resin), Mehlich-1 (PM1) and Mehlich-3 (PM3). Inorganic P (PiOH), organic P (PoOH), and calcium-bound P (PHCl) fractions. Degree of P saturation by Mehlich-3 (DPS_{M3})

Table S5 Biological and biochemical properties of the soil regarding soil dilution factors (% of soil) and microbiological activity (SA – autoclaved soil and SNA – non-autoclaved soil)

Dilutions	MBC		BSR		qCO_2	
	------- µg C g^{-1} -------		------- mg CO_2 g^{-1} h^{-1} -------		------ g C-CO_2 μg^{-1}CBM h^{-1} ------	
% W/W	AS[4]	NAS	AS	NAS	AS	NAS
25	18.5 Ba[6]	30.5 Ad	2.3 Ac	2.4 Ab	137.1 Aa	79.4 Ba
50	55.2 Ba	85.5 Ac	2.7 Aa	2.1 Bc	50.3 Ab	25.0 Bb
75	51.4 Ba	132.4 Ab	2.5 Ab	2.5 Aab	49.1 Ab	18.7 Bbc
100	77.7 Ba	238.5 Aa	2.5 Ab	2.6 Aa	32.7 Ac	11.1 Bc

Dilutions	Arylsulfatase		β-glucosidase		Acid phosphatase		FDA	
	---------- µg PNF g^{-1} h^{-1} ----------						----- mg F g^{-1} day^{-1} -----	
% W/W	AS	NAS	AS	NAS	AS	NAS	AS	NAS
25	22.8 Bb	40.5 Ac	10.9 Ba	39.7 Ad	3.6 Bb	45.8 Ad	23.5 Bc	28.4 Ac
50	21.4 Bb	103.1 Ab	14.2 Ba	72.4 Ac	20.4 Bab	127.5 Ac	29.4 Bb	35.1 Ab
75	20.1 Bb	117.6 Ab	16.1 Ba	99.1 Ab	31.5 Bab	177.8 Ab	33.1 Aab	33.2 Ab
100	55.1 Ba	141.5 Aa	16.3 Ba	131.5 Ab	55.4 Ba	279.3 Aa	36.2 Ba	41.5 Aa

AS: autoclaved soil and NAS: non-autoclaved soil. 25, 50, 75, and 100: soil/sand ratio % (W/W). MBC: Microbial biomass carbon. BRS: Basal soil respiration. qCO_2: Metabolic quotient. FDA: Hydrolysis of diacetate. Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ from each other using the Tukey test at ($p \leq 0.05$)

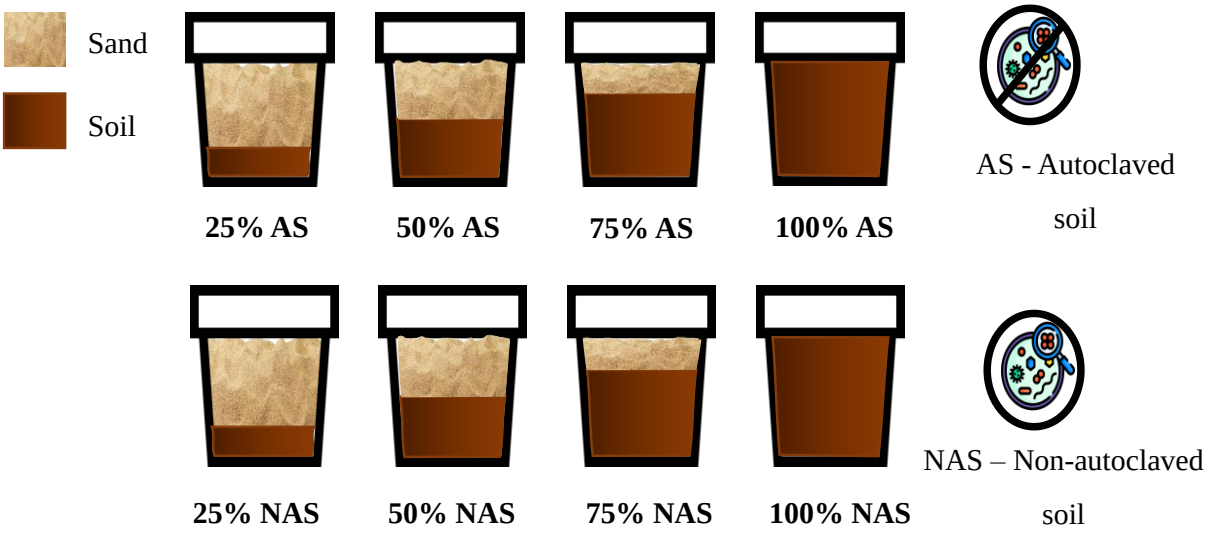

Fig. S1 Treatments with different proportions of soil/autoclaved sand (% W/W) used in the soil dilution/autoclaving experiment. AS: autoclaved soil and NAS: non-autoclaved soil. 25, 50, 75, and 100: soil/sand ratio % (W/W)

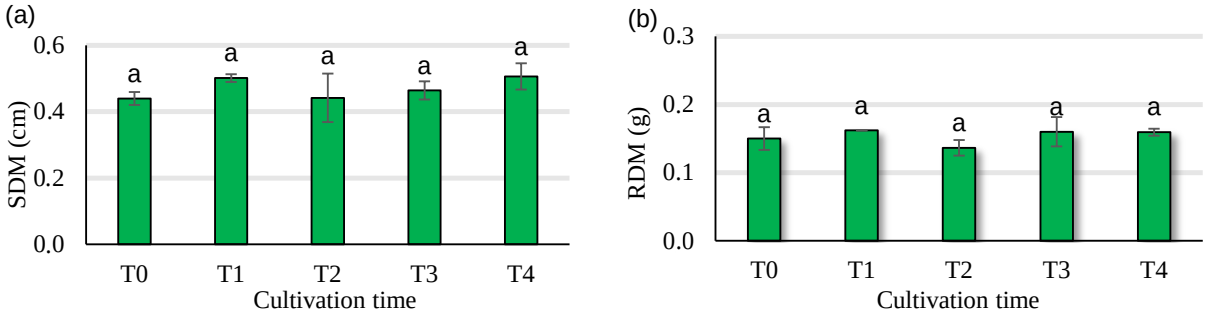

Fig. S2 Growth of cotton plants cultivated in soil samples with different cultivation histories (cultivation time experiment). Shoot (a) and root (b) dry matter productions. T0: soybean/maize cultivation; T1: soybean/cotton cultivation (first year); T2: soybean/cotton cultivation (second year); T3: soybean/cotton cultivation (third year); T4: soybean/cotton cultivation (fourth year). Columns with the same letters do not differ by Tukey test ($p \geq 0.05$)

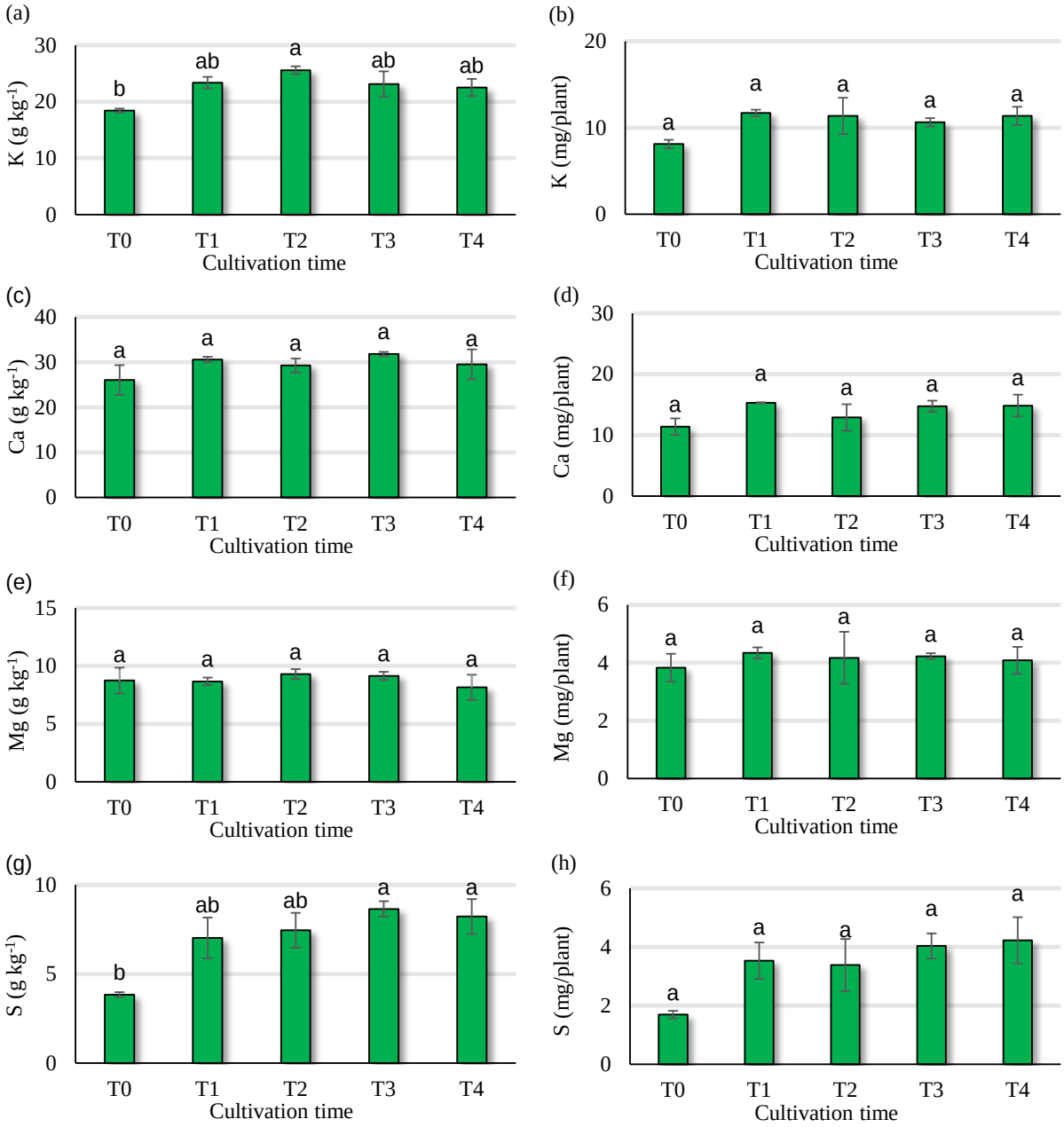

Fig. S3 Nutritional status of cotton plant cultivated in soil samples with different cultivation histories (cultivation time experiment). Concentration and accumulation of K (a and b), Ca (c and d), Mg (e and f), and S (g and h) in leaves. T0: soybean/maize cultivation; T1: soybean/cotton cultivation (first year); T2: soybean/cotton cultivation (second year); T3: soybean/cotton cultivation (third year); T4: soybean/cotton cultivation (fourth year). Columns with the same letters do not differ by Tukey test ($p \geq 0.05$)

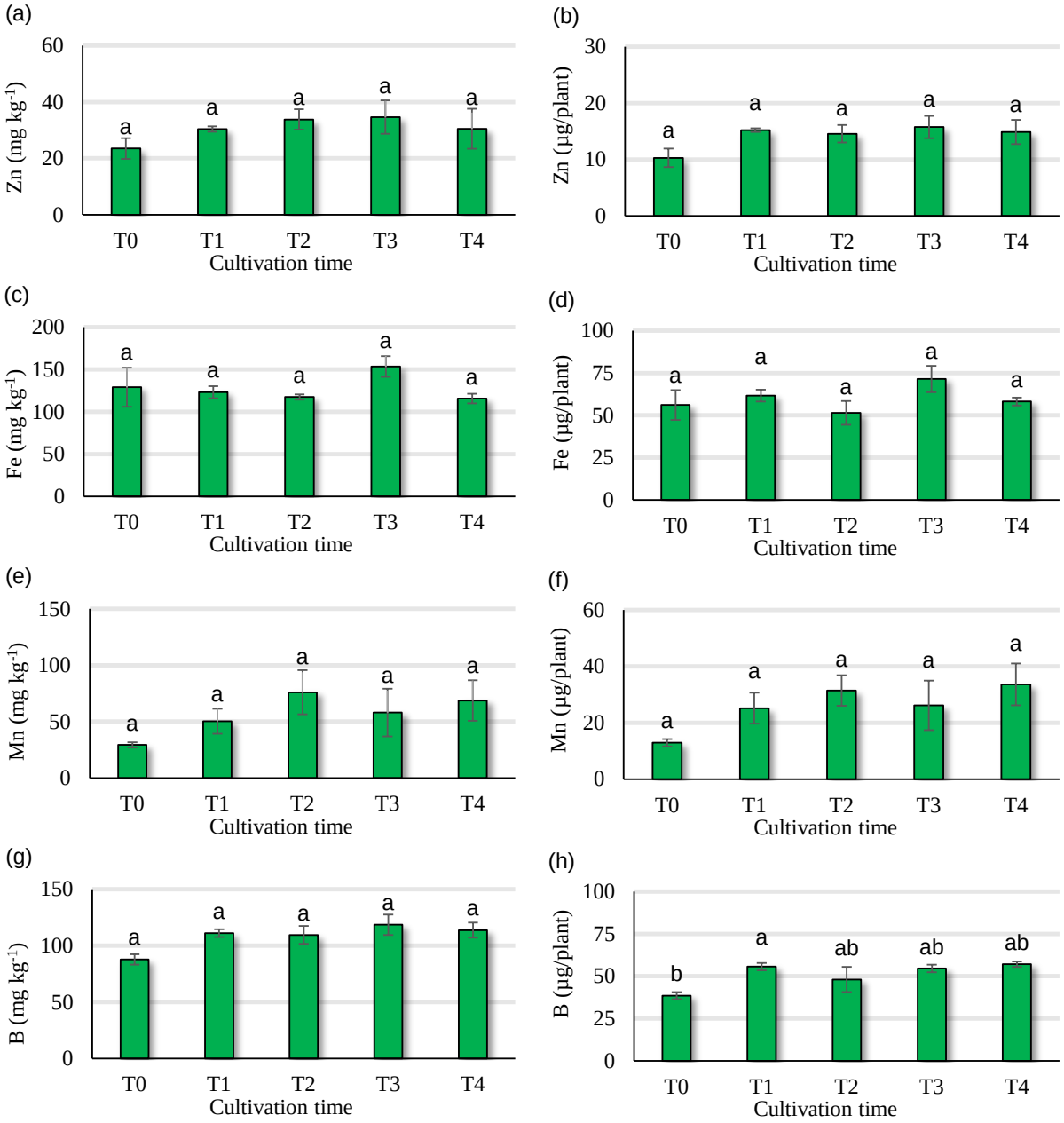

Fig. S4 Nutritional status of cotton plants cultivated in soil samples with different cultivation histories (cultivation time experiment). Concentration and accumulation of Zn (a and b), Fe (c and d), Mn (e and f), and B (g and h) in leaves. T0: soybean/maize cultivation; T1: soybean/cotton cultivation (first year); T2: soybean/cotton cultivation (second year); T3: soybean/cotton cultivation (third year); T4: soybean/cotton cultivation (fourth year). Columns with the same letters do not differ by Tukey test ($p \geq 0.05$)

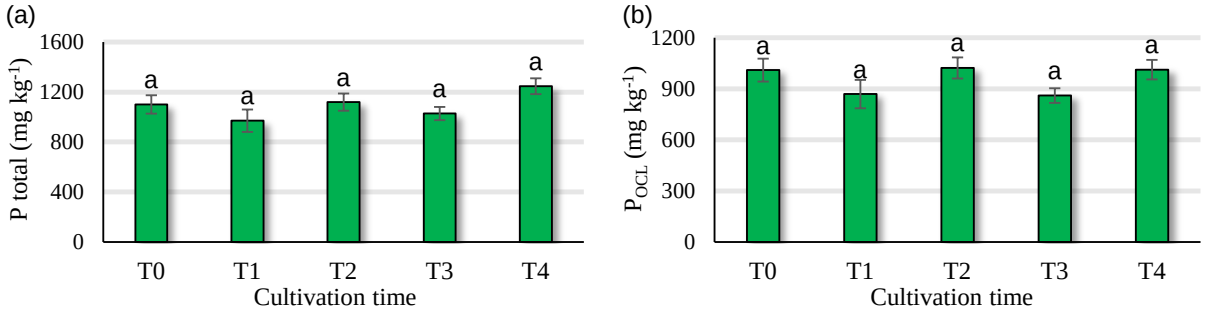

Fig. S5 Total P (a) and occluded P (b) fractions in soil samples with different cultivation histories (cultivation time experiment). T0: soybean/maize cultivation; T1: soybean/cotton cultivation (first year); T2: soybean/cotton cultivation (second year); T3: soybean/cotton cultivation (third year); T4: soybean/cotton cultivation (fourth year). Columns with the same letters do not differ by Tukey test ($p \geq 0.05$)

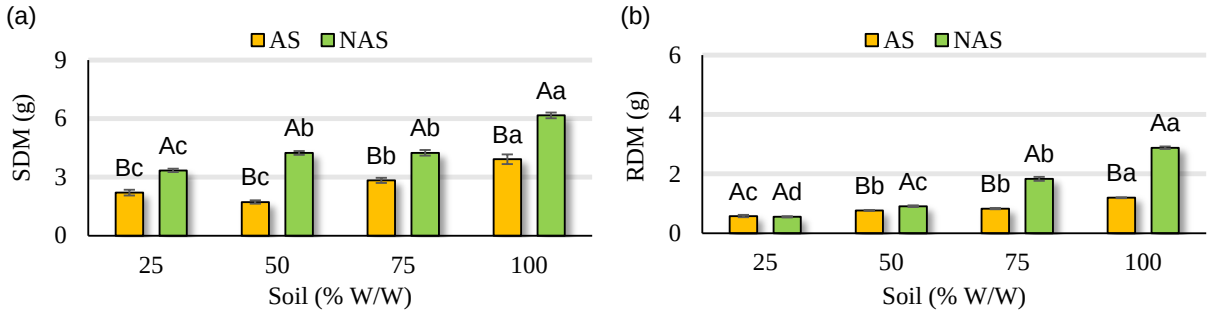

Fig. S6 Growth of cotton plants cultivated in different proportions of soil/autoclaved sand (soil dilution/autoclaving experiment). Shoot (a) and root (b) dry matter productions. AS: autoclaved soil. NAS: non-autoclaved soil. 25, 50, 75, and 100: soil/sand ratio % (W/W). Uppercase letters compare microbiological activity conditions (AS and NAS) in each dilution, and lowercase letters compare dilutions in each microbiological condition by Tukey test ($p \leq 0.05$)

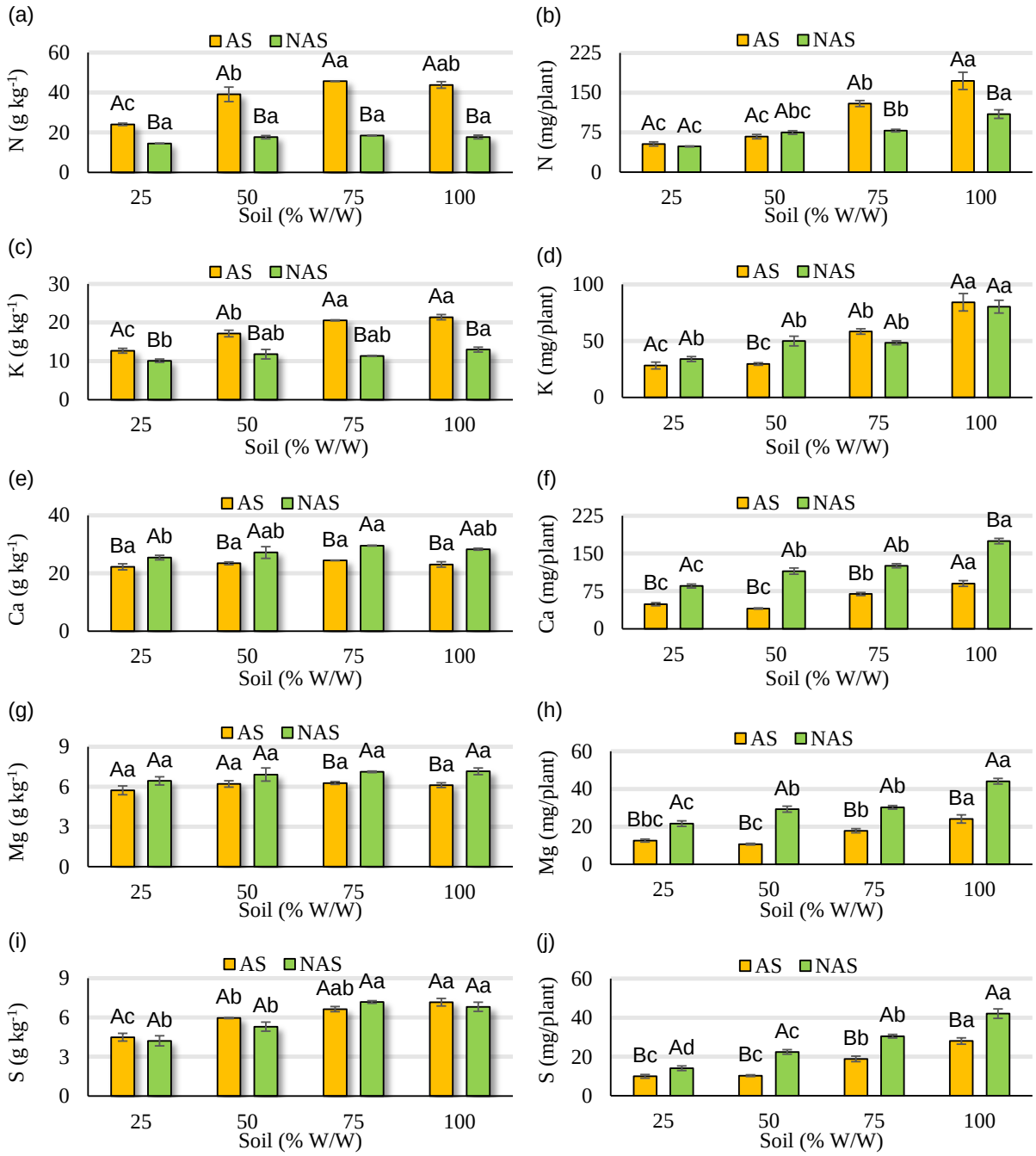

Fig. S7 Nutritional status of cotton plants cultivated in different proportions of soil/autoclaved sand (soil dilution/autoclaving experiment). Concentration and accumulation of N (a and b), K (c and d), Ca (e and f), Mg (g and h), and S (i and j) in leaves. AS: autoclaved soil. NAS: non-autoclaved soil. 25, 50, 75, and 100: soil/sand ratio % (W/W). Uppercase letters compare microbiological activity conditions (AS and NAS) in each dilution, and lowercase letters compare dilutions in each microbiological condition by Tukey test ($p \leq 0.05$)

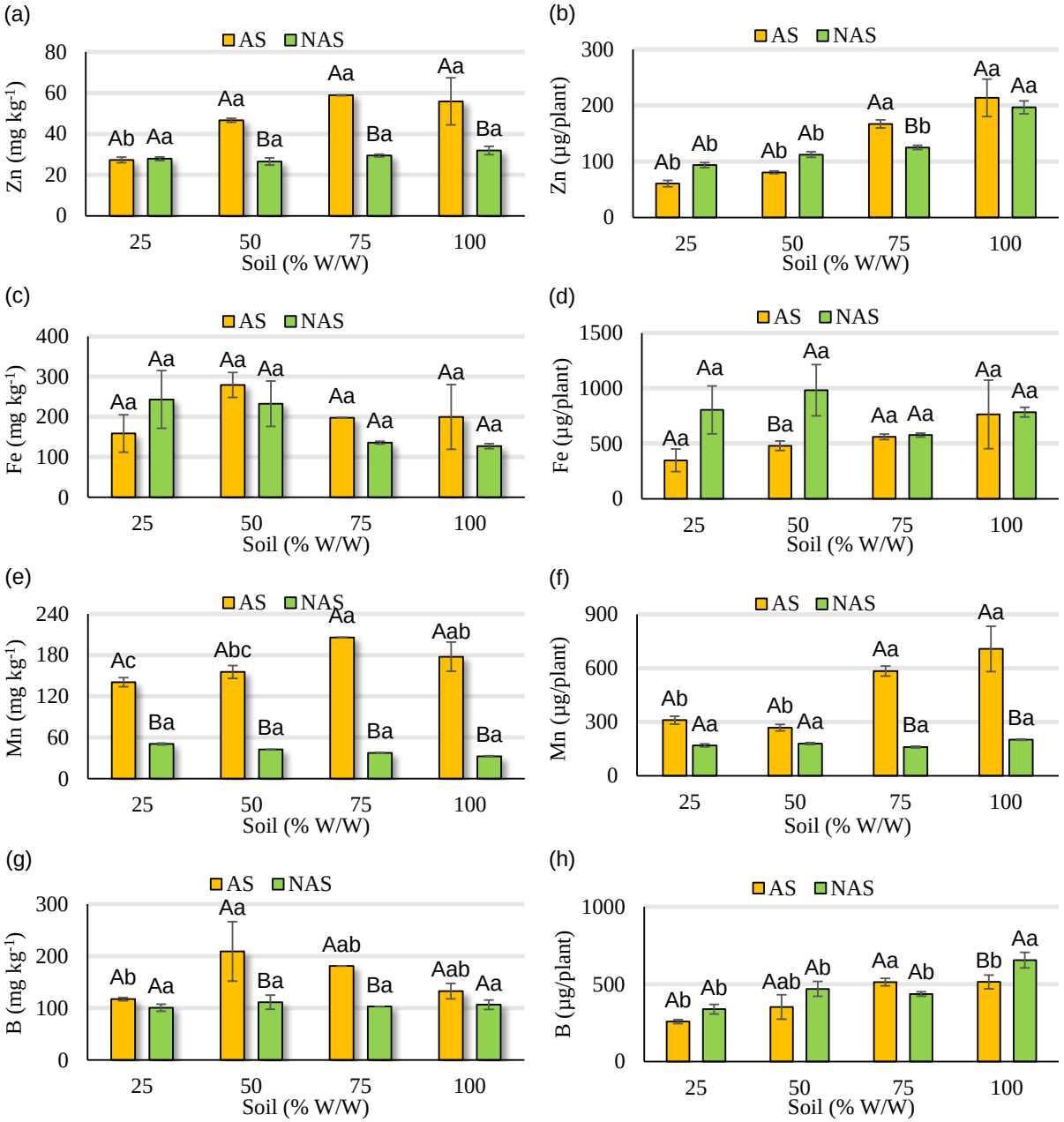

Fig. S8 Nutritional status of cotton plants cultivated in different proportions of soil/autoclaved sand (soil dilution/autoclaving experiment). Concentration and accumulation of Zn (a and b), Fe (c and d), Mn (e and f), and B (g and h). AS: autoclaved soil. NAS: non-autoclaved soil. 25, 50, 75, and 100: soil/sand ratio % (W/W). Uppercase letters compare microbiological activity conditions (AS and NAS) in each dilution, and lowercase letters compare dilutions in each microbiological condition by Tukey test ($p \leq 0.05$)

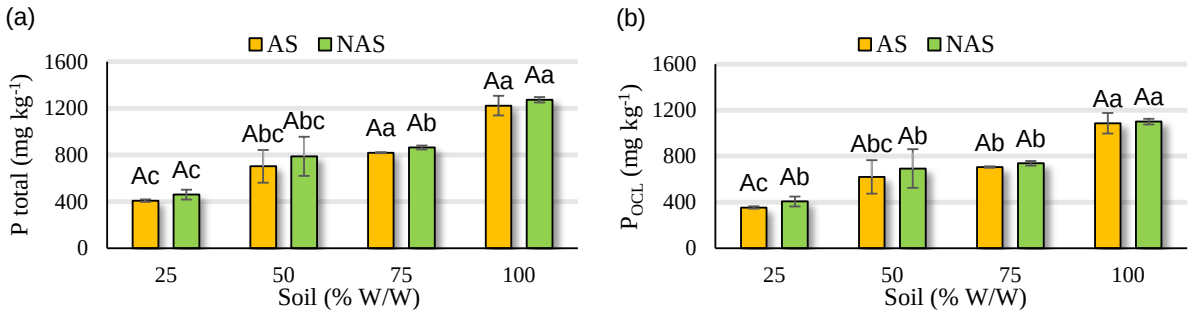

Fig. S9 Total P (a) and occluded P (b) fractions in different proportions of soil/autoclaved sand (soil dilution/autoclaving experiment). AS: autoclaved soil. NAS: non-autoclaved soil. 25, 50, 75, and 100: soil/sand ratio % (W/W). Uppercase letters compare microbiological activity conditions (AS and NAS) in each dilution, and lowercase letters compare dilutions in each microbiological condition by Tukey test ($p \leq 0.05$)

Hydroponic experiment

The hydroponic experiment was carried out to determine the critical level of P (Fig. S10c) in the leaves of cotton seedlings since P was the most affected nutrient in plants in soil experiments. The experimental design was completely randomized, with five treatments and three replications. The treatments comprised four P concentrations in the nutrient solution (0.25, 0.5, 1.0, and 1.5 mM P). Each experimental unit consisted of a pot with two plants. The experiment was carried out in a greenhouse. Cotton seeds were germinated in a polypropylene tray with autoclaved sand and irrigated with Hogland's nutrient solution (10% ionic strength). Seven days after sowing, the seedlings were transplanted into polypropylene pots (0.8 L capacity) filled with Hoagland nutrient solution (100% ionic strength) and modified according to the treatments (Table S6). Plants were harvested 35 days after transplanting.

Table S6. Nutrient solution composition for the hydroponic experiment.

P	KH_2PO_4 (1 M)	KCl (1 M)	KNO_3 (1 M)	$Ca(NO_3)_2$ (1 M)	$MgSO_4$ (1 M)	Micronutrient solution	Fe-EDTA
-- mM --	----------	----------	----------	---------- mL L^{-1} ----------	----------	----------	----------
0.25	0.25	1.75	5	5	2	1	1
0.50	0.50	1.50	5	5	2	1	1
1.00	1.00	1.00	5	5	2	1	1
1.50	1.50	0.50	5	5	2	1	1

Micronutrient solution: H_3BO_3 (2.86 g L^{-1}); $MnCl_2 \cdot 4H_2O$ (1.81 g L^{-1}); $ZnCl_2$ (0.10 g L^{-1}); $CuCl_2$ (0.04 g L^{-1}); and $H_2MoO \cdot 4H_2O$ (0.02 g L^{-1}). Fe-EDTA: 24.9 g L^{-1} $FeSO_4 \cdot 7H_2O$ and 26.1 g L^{-1} disodium EDTA diluted in 286 mL NaOH (1 M). Basic nutrient solution: 15 mM N; 7 mM K; 5 mM Ca; 2 mM Mg; 2 mM S; 46 µM B; 0.3 µM Cu; 88 µM Fe; 9.1 µM Mn; 0.1 µM Mo; and 0.7 µM Zn. The doses were chosen based on the work of Santos et al. (2019) and Santos et al. (2021). The nutrient solution was changed every two weeks and watered daily with deionized water. The plants were harvested 40 days after transplanting.

Regression equations were adjusted for the variables analyzed (TDM - Total dry matter e P concentration) as dependent on the P doses. The dose for maximum efficiency was obtained by deriving the adjusted equation for the TDM as a function of the P doses, considering only

95% of the maximum efficient production. The critical level of P in plants was calculated from leaf content at the P dose corresponding to maximum efficiency (Fig. S10 (c)).

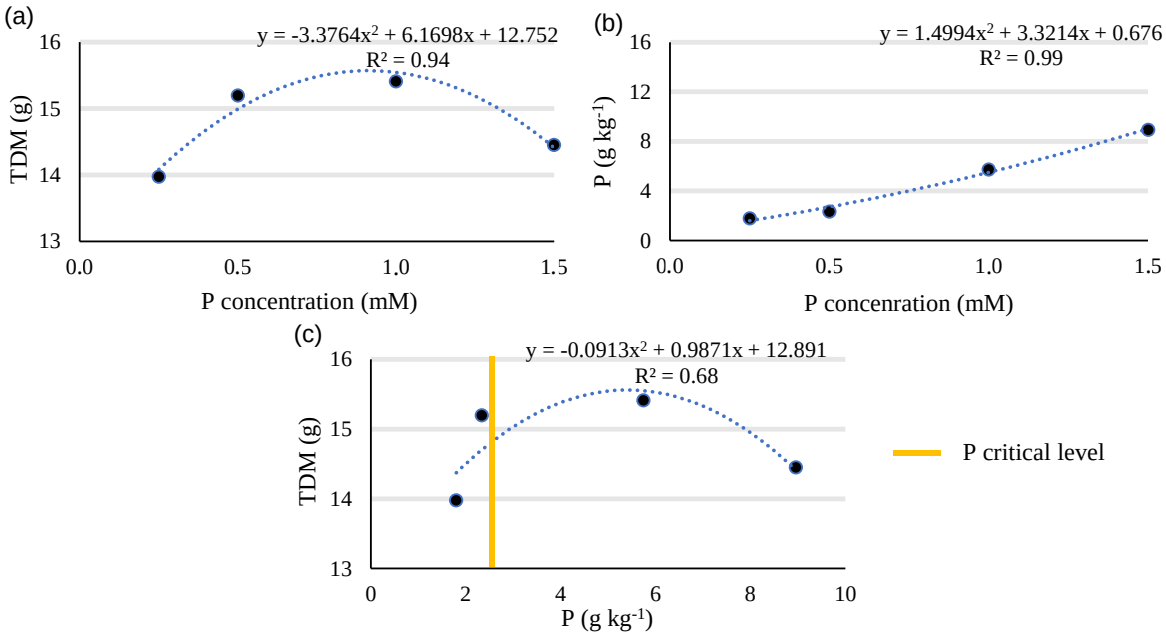

Fig. S10 Cotton plants cultivated in nutrient solution under different P concentrations (hydroponic experiment). Total dry matter production (TDM, a). P concentration in leaves (b). P critical level in cotton leaves (c). The P critical level in cotton leaves (2.6 g kg^{-1} P) was calculated as the P concentration in leaves that corresponds to 95% of maximum TDM production