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Research Article

Faba bean (*Vicia faba* L.) in natura mycorrhizal status evaluation and broad bean cropland soil biological fertility

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Abstract

The Broad bean (*Vicia faba* L.) is a legume with many virtues, commonly cultivated in the Mediterranean region, especially in Algeria, valued for its high nutritional properties and role in crop rotation systems. Moreover, as a natural green fertilizer, establishing symbiotic relationship with arbuscular mycorrhizal fungi found in the soil, faba bean brings significant agronomic benefits, promotes biodiversity and contributes to soil preservation by limiting erosion. This study was conducted in a faba bean cropland site in the northwestern region of Algeria (Sebkha-Wilaya of Oran) in 2022 to assess in natura plant roots mycorrhizal colonization, soil characteristics and its biological fertility through mycorrhizal soil infectivity (MSI) and the spore's abundance estimation. Physical and chemical properties analysis showed that the soil had a fine loamy-clay texture, with an alkaline pH, and low phosphorus content. It was moderately poor in organic matter and total nitrogen with less than 12 C/N ratio. In natura *Vicia faba* L. root fragments mycorrhizal colonization rate was very high (100%) with a highly mycorrhizal intensity (80.45%) and an arbuscular structures abundance (99.28%). Spores extracted from rhizosphere soil sample density were 1657±15.09 spores/100 g. Furthermore, results showed that *Vicia faba* rhizosphere cropland soil was high mycorrhizal infectivity was high with 2.36±1.02g MSI Units/100 g. All results strongly suggested that Sebkha *Vicia faba* cropland soil has a good biological fertility with a significant spore density. These results support the characterization of the soil as a potential biofertilizer.

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1. Introduction

The Broad bean (*Vicia faba* L.) is a nutritional, economic and ecological value legume in Mediterranean basin region semi-arid areas (Pasqualone et al., 2020; Ruissi et al., 2017) and among the main legumes grown in Algeria (Hadou el hadj et al., 2022). *Vicia faba* L. has the ability to associate with nitrogen-fixing bacteria and mycorrhizal fungi (Sánchez-Navarro et al., 2020). Among soil microorganisms, arbuscular mycorrhizal fungi (AMF) are considered essential key components of sustainable soil-plant systems, particularly in semi-arid and arid ecosystems (Benkhoua et al., 2017). They establish symbiotic relationships with the majority of terrestrial plants across various ecosystems (Bonjean & Fortin, 2023; Kalamulla et al., 2022; Ziane et al., 2021). This association promotes the plant's photosynthetic rate, water balance (Andrango et al., 2016) and plays an important role in promoting sustainable agriculture (Hayman, 1986; Hussain et al., 2024; Prin et al., 2017). AMF improve nutrient absorption, soil structure and reduce some environmental stresses (Etesami & Glick, 2020). Indeed, given mycorrhiza's importance in the different ecosystems, this research sights to emphazise the soil natural fertility under AMF and broad bean association. Thus, soil mycorrhizal potential or inoculum potential (IP) as an indicator of propagule density and mycorrhizal activity in the soil allows the quality and infectivity of soil inoculum evaluation and is used as a biological indicator (Andrango et al., 2016). Soil fertility was determined by its Mycorrhizal Soil Infectivity (MSI) estimation as well as the AMF spore's abundance.

2. Materials and methods

2.1 Study site

This study was conducted south west of Oran city (in North-Wesrtern Algerie, on a one hectare broad bean agricultural plot previously cultivated with watermelon, near Lake (Sebkha) in 2022. The study area was geographically between 35°35'39" North and 0°43'52" West, at 86 m altitude (Figure 1), characterized by a semi-arid Mediterranean climate. The summer and winter average temperatures are respectively 25 °C and 11 °C and annual rainfall between 200 to 400 mm/year.

Figure 1. Sebkha (Oran, Algeria) site localization map

2.2 Soil physical and chemical analysis

Soil samples were collected between the bean furrows, on a transect, including fifteen one-meter spaced sampling points at 0-30 cm depth. They were homogenized and then sieved (2 mm mesh), to obtain a soil composite sample for physicochemical analysis undertaken in triplicate at the agronomic analysis laboratory for fertilizers of Algeria FERTIAL (Arzew, Oran).

2.3 Broad bean in natura mycorrhization parameters determination

Faba bean root samples collection was carried out between the end of November and the beginning of December matching plants flowering. To highlight endomycorrhiza in natura, 5 cm to 10 cm long fresh roots were randomly sampled, washed with tap water and carefully fragmented into one-centimetre segments, clarified, stained according to the method described by Phillips and Hayman (1970) and then observed with Olympus light microscope to determine *in situ* mycorrhizal colonization. It was assessed with "Mycocalc"(https://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html), a calculating computer program, according to Trouvelot et al. (1986) where, the frequency of mycorrhizae in total roots (F%), intensity of mycorrhizal colonization in total roots (M%), intensity of mycorrhizal colonization in colonized roots (m%), arbuscule abundance in total roots (A%), and arbuscule abundance in colonized roots (a%) were estimated.

2.4 Mycorrhizal soil infectivity (MSI) and spore density estimation

Fifteen rhizosphere soil samples were randomly taken, mixed, homogenized and then stored at 4 °C for MSI and spores density determination. The Mycorrhizal Soil Infectivity (MSI) was determined according to the biological assay principle (Plenchette et al., 1989) using mycotrophic trap plantlets in range of concentrations of natural soil diluted with the same disinfected soil. Six dilutions of each soil samples were carried out by mixing the original soil in various quantities with the same autoclaved soil (120°C, 20 min) as 100, 48, 24, 12, 6 and 3%, (w:w) to give a gradient of concentrations. There were 5 replicates per dilution. Seeds of corn (*Zea mays* L.) were pre-germinated for two days in Petri dishes on humid filter paper. Ten germinated seeds were transplanted into plastic pots (5.5 cm diameter;10 cm high) filled with 100 g of each dilution and placed in a tissue culture chamber (25°C, 8-h photoperiod) and watered daily with sterile water. After 2 weeks culture, the entire root system of each seedling was collected, gently washed under tap water, clarified in 10% KOH for 30 min at 90°C and stained with 0.1% Trypan blue in lactophenol (Phillips & Hayman,1970). Each entire root system was mounted on a microscope slide and checked at a 400×magnification for the presence of endomycorrhizal structures. A single arbuscular mycorrhizal hyphal entry was considered as a mycorrhizal infection to give an all or nothing quantitative response. The infected plants were counted and the results were expressed as percentages of mycorrhizal plants per pot. For each soil treatment, the percentage of mycorrhizal plants was plotted against the logarithm of undisinfected soil concentration. Regression curves (model Y = BX + A). The Mycorrhizal Soil Infectivity (MSI) unit was calculated using a regression line equation and defined as the minimum dry weight (g) of soil required to infect 0% (MSI_{50}) of a plant population under the bioassay conditions and calculated for Y = 50%.

To complete the soil AMF evaluation, spores (present in the rhizospheric soil of broad bean) were extracted as reported by Gerdemann and Nicolson (1963). About 100 g of soil (in triplicate) were mixed in one litter of distilled water and homogenized using mechanical agitation for three times and sieved through a series of meshes having 500, 250 and 50 µm sieve diameters after rinsing the sieves, residue from each sieve was recovered and mixed in a 60% sucrose solution, then a density gradient created by centrifugation at 3000 rpm; the supernatant meshed and recovered AMF spores were sited in Petri plates and observed and counted under a stereomicroscope (20× magnification). A Principal Component Analysis (PCA) was performed using data from

all study soil mycorrhizal profile parameters (MSI_{50}, number of spores) and chemical parameters of the soil using Statistica 7.1 software.

3. Result and discussion

3.1 Soil physical and chemical properties

Soil physical and chemical analysis results showed that the soil has a fine loamy-clay texture, with an alkaline pH, unsalted and non-calcareous, with a low content of available phosphorus, moderately low in organic matter and total nitrogen (Table 1).

Table 1. Physical and chemical soil properties

Components	Value
Sand	12
Clay %	40
Silt %	48
Soil pH	8.28±0.02
Electrical conductivity(dS/m)	1.67±0.0
Organic matter (OM) (%)	2.65±0.03
Total limestone (%)	1.58±0.02
Limestone assets (%)	2.51±0.01
Total carbon (C)	1.58±0.02
Organic carbon (OC) (%)	1.53±0.02
Total nitrogen (N) (%)	0.15±0.00
C/N (%)	10.21±0.14
Total phosphorus (P) (ppm)	0.37±0.00
Available potassium (K) (ppm)	29.08±0.16
Available sodium (Na)(ppm)	63.44±0.90
Available magnesium (Mg) (ppm)	49.91±0.63
Available calcium (Ca) (ppm)	187.31±4.62

The loamy-clay soil texture, beneficial to AMF propagation and enhanced water retention. (Diouf et al., 2019; Plenchette, 2000). Mycorrhizal fungi are an ecological key element in the processes of improving the soil's physical, chemical and biological properties (Boyno et al., 2023). According to Brundrett (1991), the richness of a soil in fungal propagules is related to soil structure, the more soil is richer with colloids, the more its fungal propagules density increases. The study soil was unsalted and had an alkaline pH (8.28±0.02) probably related to its limestone content. Mycorrhizal fungi in the soil are highly sensitive to variations in pH (Fortin et al., 2008). It plays an important role in the availability of nutrients for plants and soil microorganisms including AMF (Bhantana et al., 2021; Van Der Heijden et al., 2015).

Soil phosphorus content was low. This element is inaccessible to plants (Etesami et al., 2021; Kour et al., 2021) due to its reactivity with certain metal complexes; it forms a complex not assimilable for the plant. The major role of AMF lies in the mobilization of poorly mobile elements in the soil, mainly phosphorus for plants (Duponnois et al., 2000), and for the development of biogeochemical cycles of soils (P, N and C) (Fall et al., 2022). AMF also promotes phosphorus uptake and transport. In addition to this, AMF can be used to improve tolerance a help the host to survive adverse climate changes and other adverse environment conditions stresses (Cheng et al, .2024). The Soil was moderately poor in organic matter and total nitrogen with a less than 12 C/N ratio, that would support a good microbial activity (Bai et al., 2023).

The data showed that the highest (156.18 ± 2.90) spore load of AMF 100 g^{-1} of soil sample that contained the lowest phosphorus content (11.1 ppm). This might be due to the need of plants to associate with phyto-beneficial microorganisms such as AMF that take part in nutrient solubilization and transportation for plants (Firdu & Dida, 2024).

3.2. Broad bean in natura mycorrhization parameters determination

In natura *Vicia faba* L. root fragments microscopic investigation showed an important root colonization by the different endomycorrhizal structures (arbuscules, hyphae and vesicles) (Figure 2 and 3).

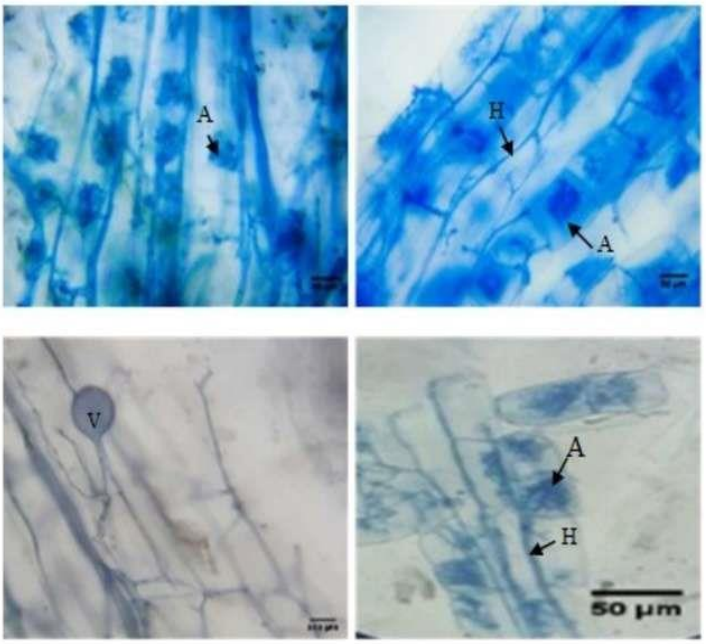

Figure. 2. Faba bean endomycorrhizal structures microscopic observation
A: Arbuscule. H: Intracellular hyphae. V: Vesicle.

These structures have been observed in various studies around the world to confirm mycorrhization in several species (Amani et al., 2023) but colonization intensity is highly variable depending on soil conditions, plant or fungal genotypes (De Oliveira et al., 2022).

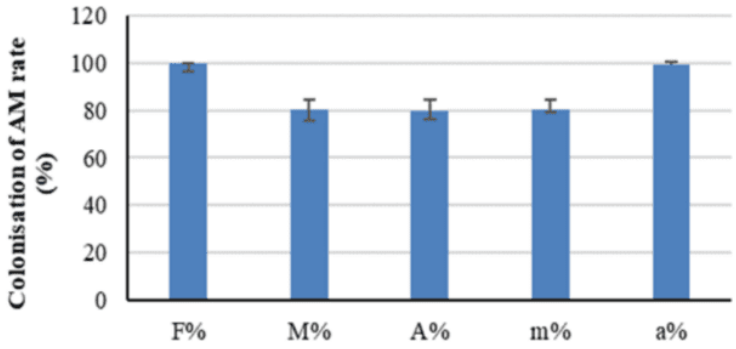

Figure. 3. Faba bean colonization by arbuscular mycorhizal fungi
Frequency of mycorrhization (F %), of root cortex colonization intensity (M %), arbuscles content of in root fragments (A %), intensity of mycorrhizal colonization mycorrhizal fragments (m %), abundance of arbuscles in the mycorrhizal parts of root fragments (a %).

Vicia faba L. natural root system mycorrhization showed an important colonization with arum type arbuscular structure. Arbuscules are the key structure for the interaction between two symbiotic partners (Yin et al., 2024). It involves the spreading of hyphae between cortical cells before they penetrate an inner cortical cell to form a terminally differentiated, highly branched structure (Luginbuehl & Oldroyd 2017; Pimprikar & Gutjahr, 2018). AMF colonization richness with arbuscules approve the mycotrophic status of this species already reported by Firdu and Dida (2024) and Pereira et al. (2019) and their presence may better reflect the effectiveness of nutrient transfer to the host (De Oliveira et al., 2022).

Most Fabaceae family species of plants and grasses are mycorrhizal (Hayman, 1983). This is the case of *Medicago sativa*, *Medicago truncatula* et *Trifolium rubens* (Saadia et al., 2024), *Retama monosperma* L. (Fakhech et al., 2020), *Accacia cyanophylla* (Dounas et al., 2022), *Retama reatam* (Mosbah et al., 2018), *Pisum sativum* L., located in Northern India (Manjula et al., 2022) and cowpea, *Vigna unguiculata* (L.), a legume cultivated in Nigeria (Diop et al., 2019).

3.3 Mycorrhizal soil infectivity (MSI)

The MSI estimation results were expressed as a percentage of non-sterile soil (Table 2). A low MSI_{50} value corresponds to a high Sebkha's soil infectivity. For rhizosphere soil under *Vicia faba*, only 2.36 ±1.02g are sufficient to infect 50 % of plants.

Table 2. Mycorrhizal soil infectivity unit (MSIU)

Parameter	Value
Regression coefficient (R2)	0.9637
MSI_{50} (MSIU) /100 g of Soil	2.36±1.02 g

There are a variety of microbial parameters that can be used as diagnostic indicators of soil quality. A number of methods have been used traditionally to assess the mycorrhizal potential to colonize a plant. These methods include: the Most Probable Number (MPN), Mean Infection Percentage (MIP) and Mycorrhizal Soi infectivity (MSI) (Andrango et al., 2016). MSI is similar to MPN method and is considered more precise; however, it requires the examination of hundreds and thousands of root system samples (Plenchette et al., 1983) and is sensitive to the environment and other variables associated to host plant and soil, host plant, soil type and substrate-inoculum proportion (Andrango et al., 2016). Several authors showed that some plant species have the ability to promote the development of fungal propagules in their rhizosphere, and can directly influence the mycorrhizal fungi spore's abundance and composition and improve the mycorrhizal soil infectivity (Bossou et al., 2019; Boyno et al., 2023; Lu et al., 2022).

A very small amount of soil was required to mycorrhizing 50 % of a maize seedling population. Duponnois et al. (2001), in the case of an herbaceous legume, showed that less than one gram of soil under *Zornia glochidiata* L., was required to colonize 50 % of a Sorghum population. The lower is MSI_{50} unit value, the higher is soil infectivity. The estimated MSI_{50} results showed a clear efficacy of rhizosphere soil mycorrhizal infectivity under *Vicia fab*a. Dejana et al., (2022) highlighted relationship between P levels and mycorrhization intensity relationship which may explain high intensity mycorrhization in the bean roots.

3.4 Spore density

The spore density evaluated as the number of spores extracted in 100 g of soil samples was 1657±15.09 spores. A varied morphotype color, shapes and size diversity was also observed (Figure 4).

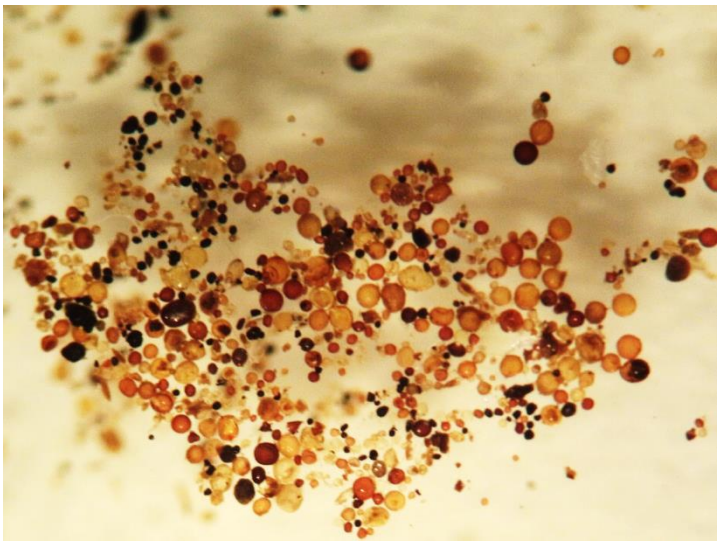

Figure 4. Morphological diversity of spores of fungal species from Sebkha site observed under a binocular magnifying glass (20 X)

The average of spores was assessed as per 100 g of soil. The wet-sieving and decanting technique is the simplest and most effective. However, in compact soils, such as clays, smaller than 40 µm spores'll be lost and should be sieved again through a cellulose filter under vacuum (Pacioni, 1992). Boyno et al. (2023) proposed new highly effective technique, to detect maximal spores Numbers in plant rhizosphere samples, using an ultrasound for AMF spores separation from soil particles which could be observed clearly and fungal spores, particularly those that had big sizes, could be easily extracted without the need for sieves. A Principal Component Analysis (PCA) was performed using data from all study soil mycorrhizal profile parameters (MSI_{50}, number of spores) and chemical parameters of the soil. The Principal Component Analysis (PCA) graphically represented the variability of biological parameters and chemical properties of the study soil. Both axes described 100% of the total variation. The first axis expresses the largest percentage of the change (61.43%) (Figure 5).

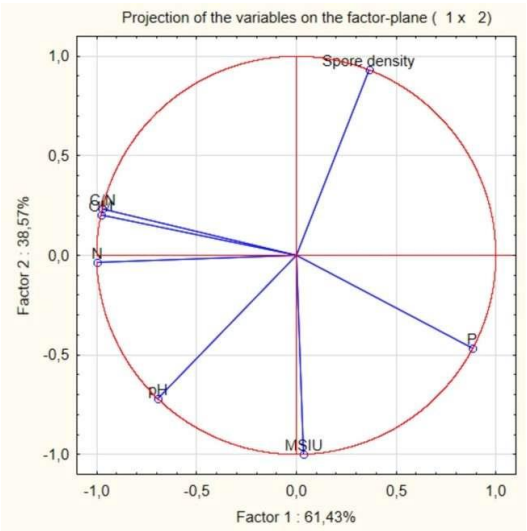

Figure 4. Principal component analysis of mycorrhizal profile parameters and soil physico-chemical parameters at the factorial level (F1 and F2).
C: Carbon, OM: organic matter, N: nitrogen, P: phosphorus, MSIU: Mycorrhizal Soil Infectivity Units

P, N, C/N and OM were represented on the F1 axis, which represents the chemical properties of the soil. Phosphorus was negatively correlated with nitrogen (r = -0.86), C/N (r = -0.99), and MO (r = -0.97). Biological fertility parameters (MSI_{50}, Spore density) and pH were represented on the F2 axis. The pH was positively correlated with MSI_{50} (r = 0.69) and negatively with spore number (r = -0.92).

The study soil under *Vicia faba* had a high spore's density (1657 spores/100 g rhizosphere soil). According to Yaseen et al. (2020), the highest spore density was found in the rhizospheric soil of the species of family Fabaceae with spore number 276, among other families. Firdu and Dida (2024) established that AMF associated with faba bean rhizosphere soil samples collected from three districts density/load that varied from 62.62 to 156.18 was obtained from 100 g of soil samples. However, the calculated density may not reflect the exact number of spores since counting moribund spores may overestimate the result. However, the distribution of spores and the mycorrhizal colonization infection rate can be affected by the soil's physical and chemical properties, host plant dependence and age, AMF sporulation capacity (Šmilauer et al., 2021) and environmental conditions (Duponnois et al., 2001; Tang et al., 2024).The measured density of spores from study site is lower than that obtained by Bossou et al. (2019), who suggested that approximately 12501.50 spores/100g of dry soil under cultivation of *Zea mays* in the different agro-ecological zones of North Benin and North Burkina Faso. The abundance of spores in the Ivorian cocoa orchard varied between 7.89 and 17.84 spores g^{-1} of soil, with an average of 13.42 spores g^{-1} of soil (Amani et al., 2023).

Under *Medicago sativa* L., *Triticum aestivum* L., *Hordeum vulgare* L., *Avena sativa* L., Sharif and Moawad (2006) enumerate more than 4000 spores/kg of low-input practice on marginal rhizosphere soil in the North-West Frontier Province region of Pakistan and an average number of spores between 1333 and 4000 spores/kg, under *Helianthus annuus* L., *Oryza sativa* L., *Phaseolus vulgaris* L., *Trifolium alexandrinum* L., *Solanum tuberosum* L., *Solanum lycopersicum* L. and *Pisum sativum* L. and with *Brachiaria precumbens* (Poaceae) plant with an average of 15.1 per 10 g soil (Suharno et al., 2017).

Conclusion

This study allowed evaluation of broad bean in natura mycorrhizal status and the AMF growing in soil richness as a good indicator of rhizosphere soil's biological fertility. The results highlighted the mycotrophic nature of the bean and the low MSI_{50} value, indicating strong biological soil fertility. The management of the mycorrhizal potential is important for the development of sustainable agriculture because AMF are the most important soil constituents of soils. AMF represent one of the major challenges for sustainable agriculture and an essential key component of sustainable soil-plant systems, particularly in semi-arid and arid ecosystems. This can only be achieved by limiting impacting factors (chemical inputs, limiting the use of fungicides, herbicides, and soil work) and implementing agronomic practices that promote the sustainability of the symbiosis. This study allowed evaluation of broad bean in natura mycorrhizal status and the AMF growing in soil richness is a good indicator of rhizosphere soil's biological fertility. The various results revealed the bean's mycotrophic nature and a low MSI_{50}, leading to good biological soil fertility. Since mycorrhizal potential in soils is affected by different factors such as host plant, soil type and substrate-inoculum proportion it is necessary to adapt an appropriate method of assessing AMF infection that is most suitable for use. A study including other bean cultivation sites is essential to confirm the mycotrophic status of the species and its impact on the biological fertility of agricultural soils. In order to promote the sustainable development of agriculture, CMAs represent a promising path that inevitably requires a questioning of traditional operating and production patterns. Hence, to extend the overall status of faba bean associated with AMF, further studies should be conducted considering different seasons and soil types. In addition, AMF

community related to *Vicia faba* identification should be improved using molecular techniques including metagenomics to reveal out the holistic status of faba bean associating AMF.

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Authors' declaration and contribution

The authors have no conflict of interest to declare. A Ch A (PhD student) conducted laboratory works, Z I (Professor Biology) supervised student work data analysis and paper redaction, AK (Biostatistics lecturer) advised about the laboratory techniques and directed results advice manuscript proofreading before submission. All authors read and approved the final version of the manuscript.

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