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Abstract

Glomalin-related soil protein (GRSP) is believed to be produced by arbuscular mycorrhizal fungi (AMF). However, this fraction of organic matter is influenced by various soil and land-use parameters and its link with AMF has been questioned. The aim of this study was to advance the understanding of the origin of GRSP and its value as a marker of AMF activity by focusing on the effects of soil P status. Archived soils from two phosphorus fertilisation field trials on sandy soils under maize cultivation in the south-west of France were studied. Trends in GRSP and soil organic carbon (SOC) were compared. Grain yield and available P (Olsen-P) were monitored and compared to assess P sufficiency/limitation. The time trends of GRSP for each site were not significant. No significant P-fertilization effect on GRSP was observed for the P-sufficient continuously cropped soil, for which the crop yield increase was small. For the P-deficient, former forest soil, P fertilization led to a marked increase in crop production and a significantly larger GRSP content. These trends are coherent with GRSP input linked to crop C-inputs, including the incorporation of crop residue.

Keywords: glomalin; Autoclaved-citrate extractable (ACE) protein; phosphate; fungal activity; long-term field trial

Glomalin-related soil protein (GRSP) is an empirically defined component of soil organic matter that has attracted much attention since it was first reported [1]. It is now recognized to be a complex mixture of proteins and non-proteins [2]. It was initially claimed to be of arbuscular mycorrhizal fungal (AMF) origin, and has been recommended as a marker of fungal activity [3, 4], however this is increasingly challenged [5-7]. Holátko et al questioned whether correlations between GRSP and AMF indices are sufficient to validate GRSP as an AMF proxy [5]. Although GRSP content is often found to increase with mycorrhizal inoculation, there is often poor correlation between GRSP content and other markers of fungal activity [5, 8-11]. Despite the importance of mycorrhizal infection for P nutrition, there have been few studies of the relation between GRSP and soil P-status. Available phosphorus (P) is known to have contrasting effects on AM fungal abundance in soil. Mycorrhizal infection and activity increase following P-fertilization of severely P-limited soil, but decrease when P is in excess [12-14]. Comparisons of soil-P and GRSP include studies of organic versus mineral fertilizers or statistical comparisons of GRSP, other AMF markers and edaphic properties of contrasting soils [4, 15-18]. A short-term pot experiment using acid-washed sand found different effects of P on GRSP and AMF [19], whereas strong effects of available P have been reported on fungal composition [10].

The aim of this study was to investigate the effect of long-term phosphorus fertilization on the GRSP fraction in order to contribute to the elucidation of the origin of GRSP. We chose to investigate archived soils from two long-term field trials of phosphorus fertilization. This allows the time trend to be established, but precludes direct measurements of other markers of fungal composition and activity, since they must be carried out using fresh soil.

Both field trials were established on sandy soils under irrigated maize crop production in the region of Bordeaux, South-West of France and arranged in randomized block designs with four replications. The crop residues were crushed and ploughed into soil (25 cm depth) before sowing. More information on the sites and trials are reported elsewhere [20, 21]. Fertilisation (including N and K to be non limiting) was applied in spring. One site, named Pierroton, was converted from forest two years before the start of the trial. For the other site, named Tartas, the soil was under continuous mixed cultivation prior to the trial. Site descriptions and treatments are summarized in Table 1. Soils were sampled in the surface layer (0-25 cm) roughly every four years before fertilisation, air-dried, sieved < 2 mm and stored until required. Archived soil from duplicate plots of two treatments were selected from each trial for the present study; zero or very low P fertilisation (P_0) or excess P-fertilisation (+P). Soil was further crushed and sieved < 200 µm prior to GRSP extraction and quantification to ensure homogeneous sampling. The C-content of this clay+silt sized fraction was about twice that of the whole (<2 mm) fraction. GRSP was extracted in triplicate according to the usual procedure (1:8 soil:solution g ml^{-1} ratio in neutral 20 mM sodium citrate solution autoclaved for 30 min at 121°C). Protein in each extract was quantified in triplicate using the nonspecific colorimetric Bradford method after recommended dilution and colour correction of the absorbance at 595 nm [22, 23]. C and N contents were quantified on the <200 µm soil samples by elemental analysis.

Analyze of variance (ANOVA) were used to investigate changes in GRSP and SOC depending on treatment and trial period. These analyses were carried out using the free software environment R [24].

Figure 1 shows for both trials, the average of data of two plots for each P-fertiliser treatment. In the cleared forest site (Pierroton), grain production declined rapidly in the absence of P-fertiliser but was immediately restored and maintained at about the initial production when limited P was supplied after 7 yr. Excess P-fertilisation (+P) gave an initial increase in grain production which then levelled off. Overall the effect of P-treatment on grain yield was highly significant ($P< 0.001$), but neither trial period nor the interaction between treatment and trial period were significant (Table SM_1). Available P increased continuously in the +P soils and decreased in the absence of P-fertilization then recovered and stabilised when sub-optimal P was applied. P-treatment, trial period and their interaction also had highly significant effects on soil-P availability ($p<0.001$, Table SM_1). In the Tartas, previously farmed site, grain production increased significantly ($P< 0.001$) and continuously for both P_0 and +P soils, with a small significant yield increase (+9%, $P<0.001$) with the +P treatment. Available P was correspondingly greater in the +P treatment ($P<0.001$) but with no time effect. C content was initially greater in the cleared forest site than the farmed site, with no evidence of C depletion following the introduction of maize cropping, in contrast to the rapid decrease often observed following deforestation in temperate zones [16–18]. The conservation of SOC may be due to the mulching with crop residue. At Tartas, there was a small, slightly significant decrease of SOC with time ($P<0.05$), but no significant P-fertilisation effect was observed.

GRSP content was greater at the previously forested Pierroton site than for the continuously farmed Tartas site, in accordance with the previously reported land-use effect of GRSP content [7]. GRSP content for the Pierroton site showed considerable inter-plot variability, especially in the early stages of the trial. ANOVA showed that both GRSP and GRSP/SOC were significantly lower in the P_0 than the +P plots ($P<0.01$). This points to GRSP input linked to C-dynamics related to crop production and residue application, rather than to fungal activity. Straw addition has been reported to enhance GRSP [25, 26]. For Tartas no coherent time trends or P-effects were apparent on C content, and there was no significant treatment or time effect on GRSP or GRSP/SOC. This suggests that SOC and GRSP may already have been at steady state for agricultural land-use at this site. The accumulation of available P prior to the trial may have inhibited fungal activity, and further, excess additions of P would therefore have had little or no impact on either primary production or fungal activity. According to the ANOVA analysis, there was no significant change in GRSP content at either site during the time of the trials (>0.05). This suggests a strong legacy effect with a relatively small shift in GRSP content in response to land management changes. The absence of a strong time effect on GRSP indicates that GRSP content results from a dynamic balance between input and turn-over.

In conclusion, GRSP content did not change markedly during the long-term (20-30 year) trials. When P fertilization was in excess with little effect on crop yield, no effect was observed on GRSP. In P-deficient soil, P fertilization increased both crop yield and GRSP content. Accounting for this trend assuming GRSP to be a product of fungal activity, would have the unlikely implication that P addition to a P-deficient former forest soil increased fungal activity. The greater GRSP content and it's enrichment within organic matter following P-addition to P-deficient soil are coherent with GRSP being directly or indirectly linked to crop production. Direct links would arise from greater root activity during growth and changes in fungal composition and activity, and indirect effects would include the stimulation of GRSP content by greater residue incorporation, as previously observed for straw. The rather small change in GRSP following land-use change (forest to cropland) suggests that GRSP is a stable fraction of SOC.

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Figure and Table Captions

Figure 1.

Grain dry mass production (a, f), Olsen P content of air-dried sieved soil (<2 mm) (b, g), SOC of air-dried sieved soil (<2 mm) (c, h), GRSP content of < 200 µm size fraction (d, i) and GRSP relative to C-content of the <200 µm size fraction (e, j) for the Pierroton deforested site (a-e) and Tartas continuously farmed site (f-j). Open Symbols indicate control (**P_0**) and closed symbols phosphorus addition (**+P**). Bars (positive or negative for clarity) indicate standard deviation between plots. Analytical standard deviation between triplicates was about 5% and standard deviation between triplicate subsamples of soils about 7%.

Table 1.

Site location, soil class and summary of P treatments for plots chosen for the study.

Figure 1

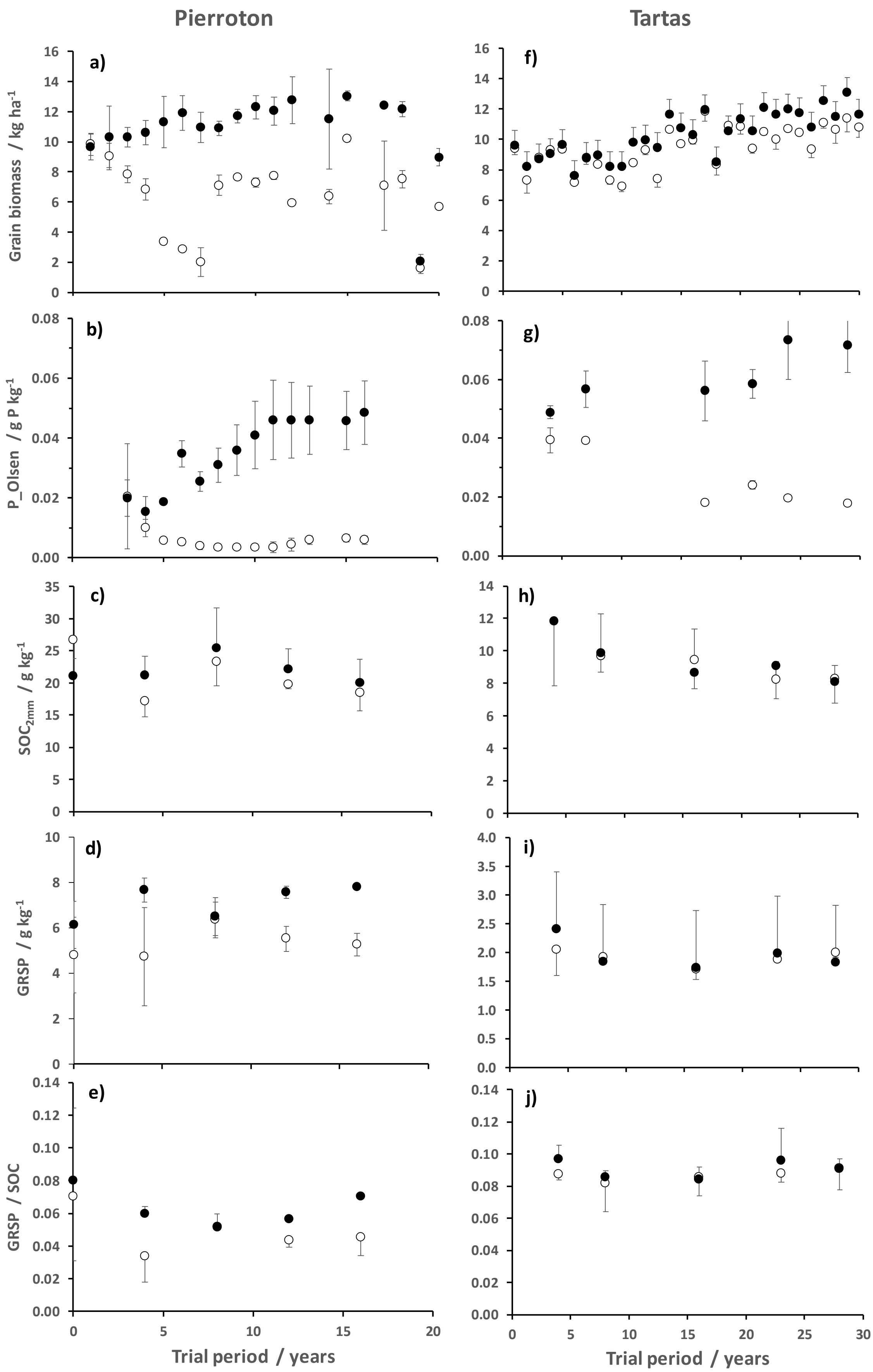
Pierroton
Tartas
a)
b)
c)
d)
e)
f)
g)
h)
i)
j)
Grain biomass / kg ha-1
P_Olsen / g P kg-1
SOC2mm / g kg-1
GRSP / g kg-1
GRSP / SOC
Trial period / years
Trial period / years

Table 1.

Site name	**Pierroton**	**Tartas**
Coordinates	44° 44’N, 0° 46’ W	43°52’N, 0°44W
Altitude / m	60	55
Mean annual temperature / °C	13.5	13.6
Mean annual rainfall / mm	950	917
Period of experiment (duration / year)	1995-2015 (20)	1972-2000 (28)
Previous crop	Pinus forest	Mixed farming, mostly maize
Soil Class : World Reference Base for Soil Ressources	Podzol	Arenosol
*P fertilisation $\mathbf{P_0}$	6.7 (0, then 10 after 7 years)	0
*P fertilisation **+P**	91 (120, then 80 after 7 years)	96
/ kg P ha^{-1} yr^{-3}		

*For the Pierroton site P additions varied over the Trial period, the average P-addition for each treatment is given and in brackets the range of additions. More information on the full trial treatments may be found in [21] for the Pierroton site and [20] for the Tartas site.

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