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Grape marc nitrogen mineralisation study

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1 Introduction

This grape marc nitrogen mineralisation study is a follow up to the report “Soil Remediation through the use of grape marc” prepared by The New Zealand Institute for Plant and Food Research Limited (Plant & Food Research) and submitted to the Marlborough Research Centre in June 2019 (Wallace et al. 2019*)*. To assist the reader in understanding the reasons why this follow-up study was conducted, much of this background information has been extracted from the June 2019 report.

This soil remediation report identified that the volume of grape marc being produced by the Marlborough wine industry has been steadily increasing over recent years. In 2018, 313,038 tonnes of grapes were harvested in Marlborough, which resulted in approximately 62,608 tonnes of grape marc to dispose of. No industry-wide strategy of sustainably disposing of the grape marc has been developed.

Research conducted by the Marlborough District Council (Oliver 2018) identified that “soil compaction, soil organic matter loss and loss of nutrients to water are significant problems for Marlborough”. There was also evidence of major reductions in soil organic matter and increases in compaction following the conversion of pasture sites to vineyard.

The opportunity for a win-win scenario where grape marc can be applied to land to form part of a strategy to remediate the declining soil quality in the Marlborough region was identified. However, disposal of grape marc to land falls under the Wairau/Awatere Resource Management Plan (WARMP). The WARMP states that the total allowable nitrogen (N) load onto land from solid waste shall not exceed 100 kg N ha^{-1} per year or a total application depth of 50 mm, measured when applied (Marlborough District Council 2009). Following these rules and working off an estimated grape marc N content of 1.2% (Deurer et al. 2000), the disposal of the 2018 grape marc load to land would require approximately 7325 hectares. Clearly, this would present a significant logistical hurdle. Fortunately the limits set in WARMP can be exceeded as an “activity not included in the plan" if the appropriate land use consent is obtained (Oliver, pers. comm., 2019). Obtaining this consent requires a peer review of the potential risks by council, and during this process detailed management and monitoring plans are sought that address the potential risks. If approval is provided, the council will then monitor the consent as part of its annual monitoring programme.

To meet the standards for a land use consent, the first step is to determine the nutrient content of the grape marc that will be disposed to land. The second consideration is to determine the rate at which this nutrient is released, and how this rate is altered by the disposal location, rate and timing. The second important component to sustainable land disposal is knowing when the measured nutrients will be released and what the effect on soil and plant properties will be. The mineralisation or release rate of nutrients from organic waste products such as grape marc is controlled by microbial processes.

Previous studies on the disposal of grape marc to land for Gisborne District Council (Deurer et al. 2000) and Marlborough District Council (Laurenson and Houlbrooke 2012) have emphasised the importance of matching nutrient release from marc to plant demand to minimise adverse environmental effects. Disposal of grape marc back to land has the potential to provide a long-term strategy to address the soil quality issues identified by Oliver (2018) while also offering a tactical solution to seasonal challenges such as irrigation restrictions (Agnew et al. 2002). Previous work has identified that the addition of organic waste to soil can increase soil porosity, reduce compaction, increase soil organic matter and increase soil water holding capacity (Eden et al. 2017).

Other work has asserted that the high phenolic content of grape marc makes it unsuitable for immediate use as a soil additive on cultivated land (Bustamante et al. 2008; Olejar et al. 2019). Other authors have observed negative effects when grape marc was used as organic fertiliser. Such negative effects are associated with an initial net immobilisation of N after the application of the grape marc compost into the soil (Bustamante et al. 2004; Flavel et al. 2005).

There is limited literature available on the effects of soil remediation using raw grape marc as the majority of work has been performed using composted marc (Kutu and Masowa 2018). This suggests that specific work is required to understand the potential of raw grape marc for remediation, as currently there is no composting facility available in Marlborough that could deal with the annual grape marc load.

The 2019 report (Wallace et al. 2019*)* identified that the use of grape marc for soil remediation in the Marlborough region shows promise as it is known that similar waste streams can be used to improve soil quality. The challenge is that in order to use grape marc as part of a long-term strategy for the improvement of soil quality in the region, we need to have a greater understanding of the rate at which nutrients and in particular N are released when marc is incorporated into soil. The first recommendation from the 2019 report was that work needs to be performed to understand the release of N from marc when it is mixed at different rates with the soils recommended as potential disposal options. This information would then allow marc incorporation rates and application frequencies to be determined that would have a negligible effect on the environment. Field trials would then be required to validate the modelled results and allow a systems approach to be taken to the disposal of grape marc. This approach would allow grape marc disposal to land to become a long-term solution for the Marlborough wine industry.

This grape marc N mineralisation study conducted in 2020 was a pilot trial using pots in a tunnel house. The trial was designed to investigate some of the questions posed in the 2019 report. The key question was to determine the rate of N mineralisation from grape marc under controlled environmental conditions. It is hoped that that this study can be followed up with field trials in order to confirm actual N mineralisation in a vineyard situation.

2 Method

2.1 Overall approach and activities

The experiment was conducted in the newly relocated and refurbished Marlborough Research Centre tunnel house. A short-term assay (4 months) was established in pots from late June until mid-October 2020 to determine the N supply from Sauvignon blanc grape marc. The assay was conducted to compare the N release from two rates of grape marc (equivalent to 100 and 300 kg N/ha) and two grape marc incorporation treatments (surface applied or incorporated in the soil). A control was also included, with no grape marc addition, in order to compare the N supply from the soil organic matter to the N supplied by the grape marc. The assay was carried out for one soil type only. A summary of the key dates is provided in Table 1.

Table 1. Summary of dates that key activities were undertaken.

Date	Description of activity
30 April 2020	Grape marc collected from Clos Henri winery
21 May	Soil collected from Rowley Crescent vineyard and sieved
24 June	Pots constructed using sieved soil, grape marc and oat seed
22 July	4-week sampling
19 August	8-week sampling
16 September	12-week sampling
14 October	16-week sampling

2.2 Grape marc collection and analyses

Fresh Sauvignon blanc grape marc was collected from Clos Henri vineyard and stored in fish bins at 3–4°C until the experiment began. Four representative samples of the grape marc were sent to Hill Laboratories for basic compost analyses soon after collection (Table 2).

Table 2. Summary of initial grape marc analyses.

Water extractable results	Average value (±SE)
pH	3.5 (±0.00)
Nitrate-N (mg/L)	10.75 (±0.75)
Ammonium-N (mg/L)	6.0 (±0.00)
Total Carbon (%)	51.45 (±0.185)
Total Nitrogen (%)	2.25 (±0.035)
C/N Ratio	23.0 (±0.408)
Dry Matter (%)	24.73 (±0.193)

2.3 Soil collection and analyses

Approximately 0.4 m^3 soil was collected from the Marlborough Research Centre Rowley Crescent vineyard headland. This soil is described in the Landcare S-Maps soil class as a Selwyn_23a.1. Well drained, deep, silt loam. The surface grass was removed prior to soil collection in order to minimise the amount of fresh plant material in the soil. The soil was collected to a depth of 15 cm. At the time of collection the soil was sieved and subsequently stored in sealed plastic sacks at 3–4°C until the experiment began. Initial soil moisture content was measured on six representative soil samples before the assay commenced. Field moist samples (1 kg) were weighed and then oven-dried at 105°C for 48 h before being re-weighed. The gravimetric water content of these samples averaged 12.5% (± 0.03% SE).

2.4 Pot assay setup

Pots were constructed on 24 June using the sieved soil (Figure 1).

Figure 1. Pots being packed with soil on 24 June using electronic scales and ruler to pack the soil in two layers by weight and depth.

Each pot contained 2856 g of field moist soil (equivalent to 2537 g of oven dry soil). As the pots had quite large drainage holes in the bottom it was necessary to line the bottom of the pots with a circular piece of weed mat in order to stop the sieved soil initially falling through the holes. This weed mat became slightly problematic in that the black oat roots became entangled in the weed mat and it took a considerable amount of time to separate all the roots from the mat when the pots were destructively sampled.

The volume of the pots when filled with soil was 2114 cm^3 and pots were packed to a bulk density of 1.2 g/cm^3. To ensure the correct bulk density was achieved, the pots were packed in two sequential layers taking into account the volume of the pot in each layer. The first layer in the bottom 7 cm of the

pot was packed with 1428 g soil and the second layer from 7 to 12.5 cm depth was packed with another 1428 g soil. There were 12 pots for each of the four grape marc treatments (i.e. four harvests x three replicates) and 24 pots for the control (i.e. four harvests x six replicates). Three composite soil samples were collected as the pots were being constructed and prior to the grape marc being added to the pots. These samples were sent to RJ Hill Laboratories Limited to determine the initial nutrient analyses of the soil (Table 3). https://www.hill-laboratories.com/analytical-testing/soil-testing/. Soil analyses conducted were: soil pH, Olsen P, exchangeable cations (K, Ca, Mg and Na), cation exchange capacity, Total Base Saturation, Volume Weight, Potentially Available Nitrogen, Anaerobically Mineralisable Nitrogen (AMN), Organic Matter, Total C, Total Nitrogen, C/N Ratio and AMN N to Total% N Ratio. Not all analyses are reported in Table 3.

The intention of the experiment was to start with soil that did not have a high amount of potentially available N, in order to observe the N being made available from the grape marc rather than being supplied from the soil. The amount of potentially available N in the soil used for the experiment was moderate (Table 3), i.e. neither very low nor high. Had a soil with high potentially available N been used then there is the possibility that the N from the grape marc may have been released faster.

Table 3. Summary of initial soil analyses.

	Average value (±SE)
pH	6.17 (±0.03)
Potentially Available Nitrogen (kg/ha)	86.7 (±2.7)
Anaerobically Mineralisable N (ug/g)	49.3 (±0.9)
Organic Matter (%)	2.4 (±0.1)
Total Carbon (%)	1.4 (±0.1)
Total Nitrogen (%)	0.14 (±0.01)
C/N Ratio	10.3 (±0.1)

Grape marc was either applied to the surface of the pots or mixed into the pots at the time of pot construction on 24 June (Figure 2).

Figure 2. Close up of Replicate 1 on 24 June on day of establishment, showing pots with two rates of surface-applied grape marc and those with either no marc or marc incorporated in the soil.

As the pots were being constructed, 20 black oat seeds were incorporated into the top 1 cm of soil. Black oats are known to be winter active and are commonly sown in the autumn in the inter-rows of Marlborough vineyards to provide a green crop that is often grazed by sheep in the winter or alternatively mown. The black oats were sown in the pots in this experiment to act as a sink crop to capture the mineral N. Twenty black oat seeds had an average weight of 697 mg, or 34.85 mg per seed. Total N concentration of the seed was 2.34%.

The initial Total N concentration of the grape marc was used to apply two equivalent field rates of N per pot; 100 kg N/ha (34 g fresh grape marc = 189 mg N on a per pot area basis) and 300 kg N/ha (102 g fresh grape marc per pot = 567 mg N on a per pot area basis).

Pots were arranged in a randomised complete block design on three tables in the plastic tunnel house.

2.5 Pot management

At the start of the experiment on 24 June 2020, 500 ml of reverse osmosis (RO) water was added to each pot to bring them up to consistent field moisture to begin the trial. As the experiment started in mid-winter and there was initially no plant material to take up water, the pots did not require any additional water in the first 3 weeks. From late July onwards the pots were watered twice a week with 100 ml of RO water and as the plants grew and temperatures increased watering was increased to three times a week with 200 ml RO water. It was necessary to apply the same volume of RO water to all pots throughout the course of the experiment, regardless of the grape marc treatment in order to not introduce watering as another treatment. However, given the relatively small volume of the pots, it was apparent that the soil moisture varied substantially over the course of 3–4 days between watering. Each pot had a saucer to capture any drainage water that ran through the pots at the time of watering. This drainage water was reapplied to the top of the pot immediately after watering to ensure there were no N leachate losses.

2.6 Measurements of growth of oats in pots

Subsequent to the establishment of the pots on 24 June, the germination and growth of the oats in the individual pots were monitored over the period that most of the oat seeds germinated in the second week of July (8, 10, 13, 15 July), to determine if there were differences between the treatments. Thereafter, the average height of all oat plants in each pot was measured prior to each of the four destructive harvests.

2.7 Pot destructive sampling

The pots were destructively sampled at 4, 8, 12 and 16 weeks after commencement of the experiment. Sampling dates were 22 July, 19 August, 16 September and 14 October, respectively. Each pot was destructively sampled into a fish bin in order to separate the soil from the roots. The coarse oat roots were able to be carefully prised loose from the soil while remaining intact to the whole plant. In all treatments some of the coarser oat roots had also grown into the weed mat placed in the bottom of the pots (Figure 3). However, these roots were able to be successfully untangled from the weed mat. The fine oat roots were sieved from the soil. In the treatments containing grape marc incorporated in the soil it was necessary to carefully separate pieces of grape marc from the roots. The plant roots were washed in tap water using a kitchen sieve in order not to lose any of the roots.

After the roots were completely free of soil and grape marc they were finally washed in RO water and dried using paper towels. All the total plant matter from each pot was weighed and then oven dried at 65°C, with dry weights after 24 and 48 hours (Figure 4). The individual dry plant samples were bagged and sent to the laboratory at Plant & Food Research, Lincoln for analyses.

After all the oat roots had been separated from the soil, a subsample of soil was collected from each bin. Grape marc from both the incorporated and surface-applied treatments were completely mixed with the soil from each pot at the time of sampling. The subsamples of soil from each pot were bagged and sent to the Lincoln lab on the overnight courier in order for them to be analysed fresh.

Figure 3. Pot being destructively harvested on 20 August (2nd sample time) showing black oat roots tangled in weed mat at bottom of pot.

Figure 4. Black oat samples from individual pots after 48 hours of drying on 24 July 2020 (1st sample time).

2.8 Measurements of air temperature, soil temperature and soil moisture

For the first month of the experiment, from 24 June to 26 July, temperature control in the tunnel house was passive through the opening and closing of the roof vents. However, it soon became apparent that solely opening the roof vents was unable to keep the temperature down below the target of 25°C, even in mid-winter. From 27 July onwards, the air temperature in the tunnel house was actively cooled by the use of a wet wall and extraction fan, with the temperature control set to try and keep the temperature below 25°C. Even with the wet wall working it struggled to keep the air temperature in the tunnel house below 25°C on warm sunny days from mid-August to mid-October. No heating was used. A single measurement of air temperature was recorded in the tunnel house every 30 seconds and output as average hourly temperature.

Soil temperature was measured in three additional pots comprised of soil and oats, but no grape marc. These pots were not part of the destructively sampled pots. Soil temperature was measured every 30 seconds and output as average hourly soil temperature.

Soil moisture was measured in the same three pots as soil temperature. However, because of delays in obtaining the soil moisture sensors, soil moisture measurements did not begin until 10 July, 15 days after the experiment began. Volumetric soil moisture was measured every 15 minutes and output as an hourly average.

2.9 Measurements of nitrogen supply

Measurements of N content of the oats and soil were carried out in the soils laboratory at Plant & Food Research, Lincoln. The dried oat samples were ground and analysed to measure the Total N content. The soil samples were sieved to pass through a 2-mm screen and analysed for soil mineral N. The results were used to calculate the amount of readily available N (in both the oats and the soil) in each pot at each sampling time point. From these results the estimates of net N supply from each of the treatments were calculated.

2.10 Data analyses

The experiment can be viewed as a 2x3x4 full factorial design with three replicates. This gave 72 data points. The 24 treatment combinations are separately randomised within each of the Reps. The treatments were:

- Method of application: Surface or Incorporated (Incorp)
- Marc rate: 0N, 100N, 300N
- Time: 4, 8, 12 and 16 weeks.

We were interested in how these treatments affect the net N supply from grape marc and other measurements. Some of the sampling was destructive so different pots were used for each measurement. For other variables we have measurements from the same pot at different times. The three replicates corresponded to groupings of pots on three separate tables within the tunnel house. For this reason, Rep is included as a random effect in the modelling. This is similar to a blocking variable.

The Marc rate of 0N corresponds to no grape marc being applied. This means the combinations Incorp:0N and Surface:0N are identical. These data are kept apart so that the effects of the method of application and rate of application can be considered separately.

Data analyses were performed using R version 3.6.3. The tidyverse suite of packages was used. The package lme4 was used to fit the (generalised) linear mixed-effects models. Modelling assumptions were checked using standard residual plots and the logarithm of the response was taken when necessary. The packages emmeans and predictmeans were used to produce the estimated marginal means and associated plots. The false discovery rate (FDR) is used to control for multiple comparisons.

3 Results

3.1 Tunnel house air temperature during the experiment

Absolute daily maximum and minimum air temperatures were recorded, from which the daily mean temperature was calculated (Figure 5). Average daily temperature in the tunnel house over the 4 months of the experiment was 14.1°C. The average daily range in air temperature was 19.3°C. The absolute maximum air temperature recorded was 32.9°C. The absolute minimum temperature recorded was 0.7°C.

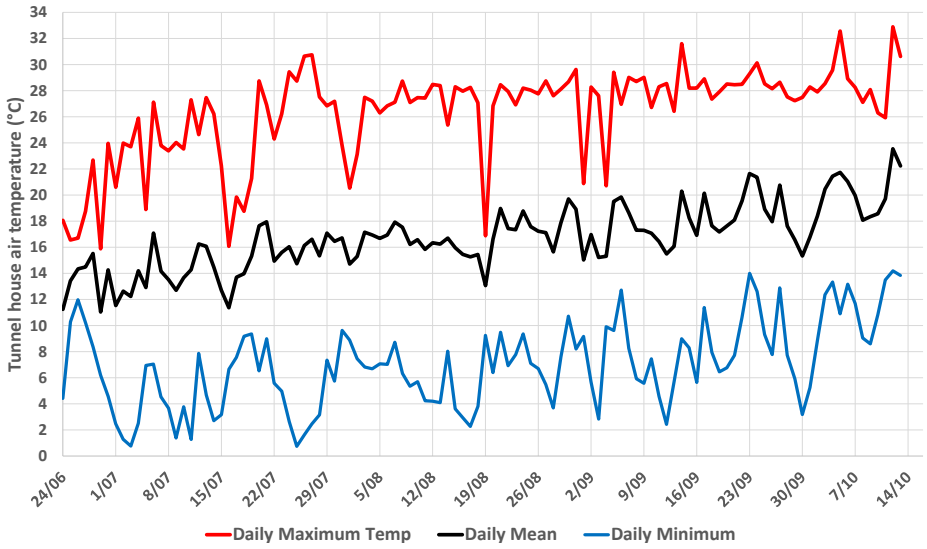

Figure 5. Air temperature in the tunnel house over the 16-week experiment.

The average air temperature in the tunnel house over the 16 weeks from 24 June to 16 October, of 14.1°C, was 3.5°C warmer than the ambient air temperature of 10.6°C over the same period at the nearby Blenheim weather station. Most grape marc from wineries becomes available from mid-March through until the end of April. If the marc was applied to vineyards in early April we would expect the average air temperature over the 4 months from April to July to be 10.3°C, which is 3.8°C cooler than the tunnel house air temperature during the experiment.

3.2 Soil temperature and soil moisture in the pots

Daily maximum and minimum soil temperatures were recorded. Average daily soil temperature (5 cm) in the three pots over the 4 months of the experiment was 14.6°C (Figure 6). The average daily range in soil temperature was 16.4°C. The absolute maximum soil temperature recorded was 39.0°C on 12 October, 2 days before the completion of the experiment. The absolute minimum soil temperature recorded was 1.1°C on 25 July. The large daily range in soil temperature (16.4°C) was mainly due to the large daily range in air temperature (19.3°C). However, the fact that the pots were only watered three times a week meant that the soil dried out a lot between watering, which gave rise to warmer temperatures in drier soil.

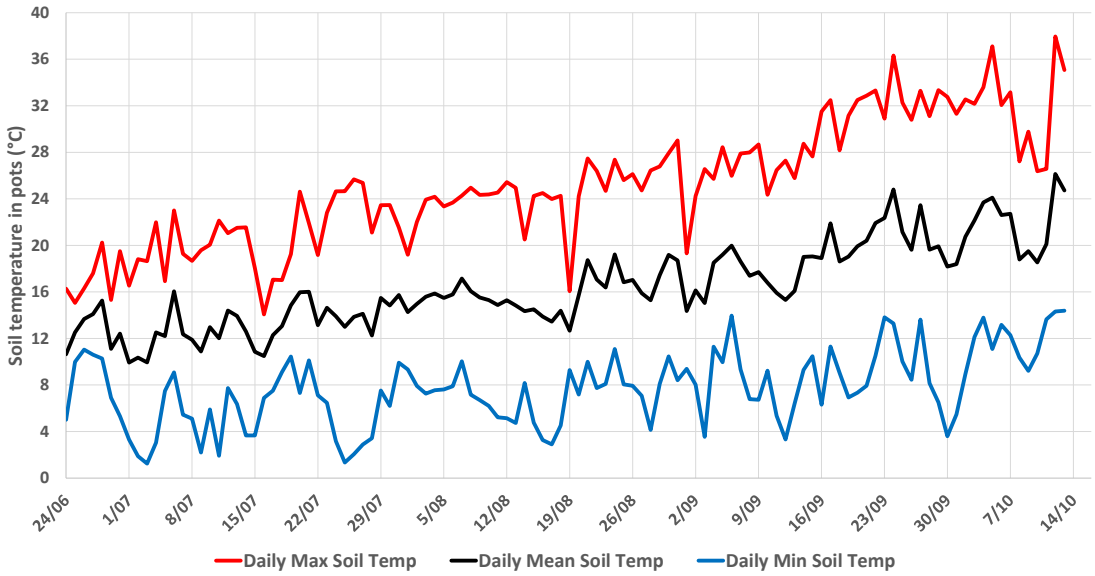

Figure 6. Soil temperature (average from three pots) over the 16-week experiment.

The average soil temperature in the pots over the 16 weeks from 24 June to 16 October was 14.6°C, which was 6.1°C warmer than the ambient 10-cm soil temperature of 8.5°C, over the same period at the Blenheim weather station. However, we would expect the average 10 cm soil temperature over the 4 months from April to July to be 8.0°C, which is 6.6°C cooler than the soil temperature in the pots during the experiment.

Average daily soil moisture (0–15 cm) in the three pots over the 14 weeks that it was monitored was 22% (weeks 3 to 16 of the experiment) (Figure 7). The average daily range in soil moisture was 4%. However, there was a marked variation in volumetric soil moisture content throughout the course of the experiment, largely because of the fact that the pots were only watered two or three times per week. The small volume of the pots and warm temperatures in the tunnel house meant that the soil was able to dry out quite rapidly.

Ambient shallow soil moisture is measured from 5 to 35 cm depth at the Blenheim weather station. The only water input the ambient soil receives is from rainfall. While this soil moisture measurement is deeper than in the pots, it is a guide as to very shallow soil moisture (0–12.5 cm) that would occur in a vineyard. Average ambient soil moisture (5–35 cm) over this time period was 34.0%, which is 12.0% higher than was recorded in the pots, over the same time period.

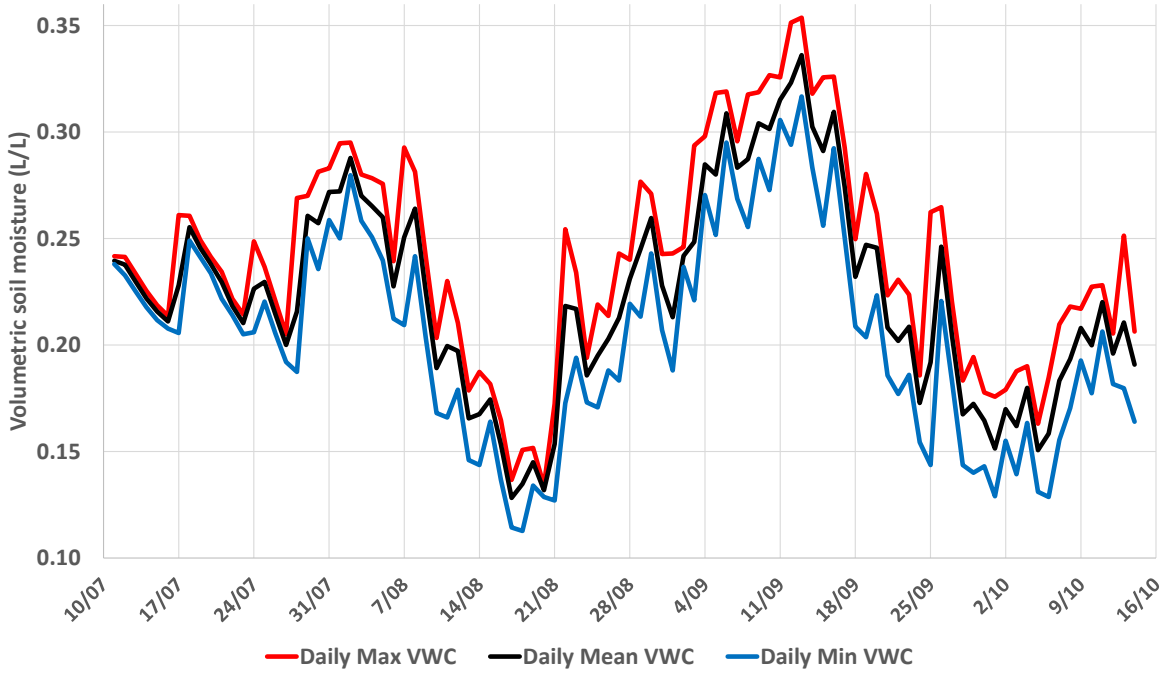

Figure 7. Volumetric water content (VWC) of the soil during the course of the experiment.

The air temperature in the tunnel house and soil temperature in the pots were considerably warmer than the corresponding temperatures that would be expected in a vineyard following grape marc application in early April. However, the soil moisture in the pots was considerably drier than would normally be expected in a vineyard over the same time period.

It is known that soil temperature and soil moisture are key factors influencing mineralisation processes and may therefore have an influence on the amount of N released from the grape marc and soil organic matter. By trying to control the air temperature in the tunnel house the aim was to try and limit the variation in soil temperature in the pots. However, in hindsight, daily watering of the pots with a smaller volume of water would have undoubtedly reduced the variation in soil temperature and soil moisture. With additional resources available it would also have been preferable to monitor soil temperature and moisture in each of the six treatments.

The pot experiment has given good indications of the N mineralisation over a 4-month period in a tunnel house. However, field trials would be necessary in order to confirm actual N mineralisation in a vineyard situation.

3.3 Germination rate and height of oats

Oat seeds were planted on 24 June and the total number of oat seeds that germinated, out of 20 seeds planted, were counted on 8, 10, 13, 15 and 22 July and the percentage germinated was calculated (Table 4). At the same time, the average height of all plants in each pot was roughly measured using a 30-cm ruler (Table 5). Individual plant height in each pot was not measured, as this would have been far too time consuming for little extra value. The measurements on 22 July were just prior to the first destructive sample on the same date, 4 weeks after the pots were established. On the afternoon prior to the second, third and fourth destructive samples on 18 August, 15 September and 13 October respectively, the average height of all oat plants in each pot was also measured.

Table 4. Germination percentage of oats over 4 weeks from date of sowing, as affected by marc treatment (0, 100, 300 kg/ha). A binomial generalised linear mixed-effects model was fitted to the data. Estimated marginal means within a row with the same letter are not significantly different (p>0.05).

Date and days from sowing	Incorporated kg/ha N			Surface kg/ha N		
	0	100	300	0	100	300
8 July – 14 days	81.8ab	77.1b	15.1c	80.2b	93.8a	32.6c
10 July – 16 days	85.5b	88.3ab	45.3c	85.2b	96.2a	76.4b
13 July – 19 days	96.0ab	93.7ab	67.6c	93.6ab	98.7a	91.8b
15 July – 21 days	98.1ab	94.8bc	72.8d	94.9bc	99.5a	92.2c
22 July – 28 days	99.5a	96.6bc	78.3d	98.8ab	99.7a	93.3c

The higher rate of grape marc had the effect of both inhibiting and delaying the germination of the oat seeds. This was most noticeable when the high rate of grape marc (300 kg/ha) was incorporated into the soil. The oats in this treatment were slower to germinate (Figure 8) and a lower percentage of the seeds germinated (Table 4). It is likely that the germination of the oats was initially inhibited by the low pH of the grape marc and the polyphenols in the grape marc. Phenolic compounds are known to inhibit seed germination and be phytotoxic to plants (Olejar et al. 2019). Where the high rate of grape marc was applied to the soil surface the oats were also slower to germinate and a lower percentage germinated. However, in this situation it is likely that the lower germination percentage and slower germination rate were due to the fact that the seedlings had to push their way through quite a thick layer of grape marc on the soil surface, as there was no grape marc in the soil close to where the seeds were germinating. There were some small positive and at other times negative effects on seed germination between the 0 and 100 kg/ha treatments, which suggests that these effects were fairly random and would be unlikely to be observed in a field situation.

Figure 8. Replicate 1 on 7 July, 2 weeks after establishment, showing already germinated oats in pots with no grape marc or low rates of grape marc, but little germination of oats in pots with high rate of incorporated or surface-applied grape marc.

Table 5. Height of oats (mm) in pots over the 16 weeks of the trial, as affected by marc treatment (0, 100, 300 kg/ha). A linear mixed-effects model was fitted to the data. Estimated marginal means within a row with the same letter are not significantly different (p>0.05).

Date	Incorporated kg/ha N			Surface kg/ha N		
	0	100	300	0	100	300
8 July[1]	58.7a	39.3b	12.3c	56.7a	52.8a	10.3c
10 July[1]	75.8a	54.2b	18.2c	76.7a	73.8a	26.4c
13 July[1]	93.3a	77.5b	43.9d	98.1a	98.3a	58.6c
15 July[1]	109.6a	95.8b	58.3d	108.8a	116.7a	76.7c
22 July[1]	155.8a	124.6b	95.8c	154.6a	156.3a	120.4b
18 August[2]	286.0ab	221.1d	267.6c	271.9bc	264.5c	298.1a
15 September[3]	289.2c	257.4d	335.9a	284.7c	284.4c	318.1b
13 October[4]	314.4c	324.8c	394.6a	324.8bc	346.8b	394.4a

[1] – Includes all 72 pots from four sampling times
[2] – Does not include pot numbers 1 to 18 from first destructive sample
[3] – Does not include pot numbers 1 to 36 from first and second destructive samples
[4] – Does not include pot numbers 1 to 54 from first, second and third destructive samples

As a direct result of the 300 kg/ha marc treatments (incorporated and surface applied) being slower to germinate, these treatments lagged behind the other treatments in their growth rate, (as measured by the average height of oats in each pot) through until 22 July, when the first destructive sampling took place. However, 4 weeks later on 18 August, at the time of the second destructive harvest, the oats in the 300 kg/ha surface-applied treatment were higher than all other treatments, except 0 kg/ha incorporated. Both the 300 kg/ha treatments had grown at a faster rate than the other treatments between the 4 and 8 weeks destructive sampling points. At the third destructive sample point on 15 September, the oats in the 300 kg/ha incorporated treatment were significantly higher than all other treatments. This treatment had grown much faster than the other treatments between the 8- and 12-week sample points. At the fourth and final harvest on 13 October, the oats in both the 300 kg/ha treatments were significantly higher than all other treatments. There was little difference in height between the 0 and 100 kg/ha treatments at the fourth harvest time point.

3.4 Crop biomass, tissue nitrogen content and nitrogen uptake

Plant biomass (g Dry Matter/pot) and tissue N content (%) were measured on the oat samples collected from each pot at 4, 8, 12 and 16 weeks after pot establishment. From these two measurements, the mass of N from each plant sample was calculated (mg N).

Table 6. Oat biomass (g Dry Matter/pot) from the surface and incorporated grape marc treatments (0, 100, 300 kg/ha) at four sampling times. A linear mixed-effects model was fitted to the data. Estimated marginal means within a row with the same letter are not significantly different (p>0.05).

Time from sowing		Incorporated kg/ha N			Surface kg/ha N		
		0	100	300	0	100	300
4 weeks	g DW/pot	1.00a	0.87a	0.42c	0.90a	1.06a	0.66b
8 weeks	g DW/pot	5.73a	2.89b	3.02b	6.05a	5.17a	5.44a
12 weeks	g DW/pot	10.77ab	6.03c	8.94b	9.43ab	9.34ab	11.46a
16 weeks	g DW/pot	13.15b	9.53c	14.82ab	12.08b	13.65b	17.54a

As was outlined in Section 3.3, seed germination and height of oats were initially inhibited by the high rate of incorporated grape marc and to a lesser extent by the high rate of surface-applied grape marc. The data in Table 6 indicate that 4 weeks after pot establishment, at the time of the first destructive pot harvest, that oat biomass of the 300 kg/ha incorporated marc treatment was significantly lower than all other treatments. Second lowest oat biomass was the 300 kg/ha surface-applied marc treatment. There were no significant differences between the 0 and 100 kg/ha marc treatments at 4 weeks. However, by the time of the fourth destructive pot sample at 16 weeks, the biomass trend had been reversed and pots with the high rates of grape marc (300 kg/ha) had the highest biomass.

Table 7. Oat nitrogen content (%) from the surface and incorporated grape marc treatments (0, 100, 300 kg/ha) at four sampling times. A linear model was fitted to the data. Model means within a row with the same letter are not significantly different (p>0.05).

Time from sowing	Incorporated kg/ha N			Surface kg/ha N		
	0	100	300	0	100	300
4 weeks	4.20a	2.93b	2.84b	4.24a	3.86a	4.10a
8 weeks	0.96c	1.13b	1.43a	0.94c	0.97c	1.18b
12 weeks	0.55e	0.79ab	0.85a	0.56de	0.63cd	0.70bc
16 weeks	0.49b	0.63a	0.63a	0.48b	0.53b	0.53b

At the 4-week sample point, the N content of the oats in the 100 and 300 kg/ha incorporated marc treatments were significantly lower than the other treatments. However, 4 weeks later, at the 8-week sample point, the trend had been reversed and the oat N content was highest in the 100 and 300 kg/ha incorporated marc treatments, and this trend was maintained at the 8- and 16-week sample points.

Tissue N concentration decreased throughout the experiment (Table 7). At the 4-week sampling point the tissue N concentrations were in the 3–5% range. At 16 weeks the tissue N concentrations were in the 0.5–0.6% range. These 16-week tissue N concentrations are very low for an oat crop (commonly 3.0–5.0%. Kay and Hill 1998), which suggests that N supply became more limiting under all treatments as the experiment progressed. This may help to explain the yellowing of the lower leaves that appeared on most of the oat plants as time progressed.

Table 8. Oat nitrogen uptake (mg N/pot) from the surface and incorporated grape marc treatments (0, 100, 300 kg/ha) at four sampling times. A linear mixed-effects model was fitted to the data. Estimated marginal means within a row with the same letter are not significantly different (p>0.05).

Time from sowing	Incorporated kg/ha N			Surface kg/ha N		
	0	100	300	0	100	300
4 weeks	41.8a	25.3b	12.0c	38.0a	40.8a	27.0b
8 weeks	55.1ab	32.6d	43.2c	56.7ab	50.1bc	64.1a
12 weeks	58.8b	47.5c	76.2a	53.2bc	58.6b	80.3a
16 weeks	64.6bc	59.7bc	92.8a	57.4c	72.3b	93.7a

Oat N uptake was a function of oat biomass and oat tissue N concentration. At 4 weeks, the oats in the 300 kg/ha incorporated marc treatment had taken up significantly less N than all other treatments (Table 8). This was probably a combination of the fact that the oats in this treatment were significantly slower to germinate than the other treatments and were therefore lagging behind in N uptake from the soil and also that N in the soil in this treatment was strongly immobilised at this early stage. However, at the 16-week sample point, the trend had been reversed and the N uptake by the oats was highest in the 300 kg/ha surface and incorporated treatments (93.7 and 92.8 mg N/pot), respectively.

3.5 Net nitrogen supply

Individual soil samples from each pot were analysed for the concentration of Nitrate N (µg/g) and the concentration of Ammonium N (µg/g). The sum of these two N concentrations gave the amount of mineral N (µg/g) in each soil sample. The mass of N in each pot was then calculated from the mineral N content and the volume of the pot.

Soil mineral N and crop N uptake were measured at 4, 8, 12, 16 weeks after grape marc application for each individual pot. In addition to these sampling points, baseline soil mineral N and seed N were measured at the start of the experiment. Using this information, several calculations were made to quantify the N supply from the different grape marc treatments. The aim being to contrast the N supply from the grape marc against the N supply from the soil itself. The background N mineralisation from the soil organic matter in the control treatment was calculated and then subtracted from the N mineralisation totals for the grape marc treatments. The calculations are summarised below:

Net N supply = [soil mineral $N_{4..16\ wk}$ + crop $N_{4..16\ wk}$] – [soil mineral N_{0wk} + seed N_{0wk}] (Eq.1)

Where:

Net N supply = mg N released per pot (2537 g DW of soil). Net supply reflected the sum of mineralisation, volatilisation, immobilisation and/or denitrification losses (leaching loses were assumed to be negligible as any drainage water was captured and re-applied to the pot surface).

$_{4..16\ wk}$ = sampling occurred at 4, 8, 12, 16 weeks (wk)

$_{0wk}$ = baseline sampling before grape marc was applied to any pots

Crop N (mg N/pot) = the amount of N contained in the crop at sampling. Crop N was calculated from crop biomass (mg/pot, above and below ground) and tissue N concentration (%N)

Soil mineral N (mg N/pot) = the amount of mineral N contained in the soil at sampling. Soil mineral N was the sum of the nitrate and ammonium components, expressed on a dry weight basis

Seed N (mg N/pot) = the amount of N contained in the seed that was sown into each pot.

Seed N was calculated from seed biomass (mg/pot) and seed N concentration (%N)

Corrected Net N supply = [Net N supply] – [Net N $supply_{control}$] (Eq. 2)

Corrected Net N supply = Net N supply (Eq.1) minus the Net N mineralisation, volatilisation, immobilisation and/or denitrification losses that occurred in the control treatments that had no grape marc added to the pots; i.e. this takes into account the N supplied from the soil during the course of the experiment

3.5.1 3-way interaction: Time by Surface or Incorporated Marc by Marc Rate

A linear mixed-effects model indicated that there was no evidence to suggest that there was a 3-way interaction between Time, Surface Incorporated Marc and Marc Rate. However, there was evidence to suggest that all three 2-way interactions were significant. This means we can discuss the effects in pairs rather than having to consider all three effects at once.

3.5.2 2-way interaction: Time by Surface or Incorporated Marc

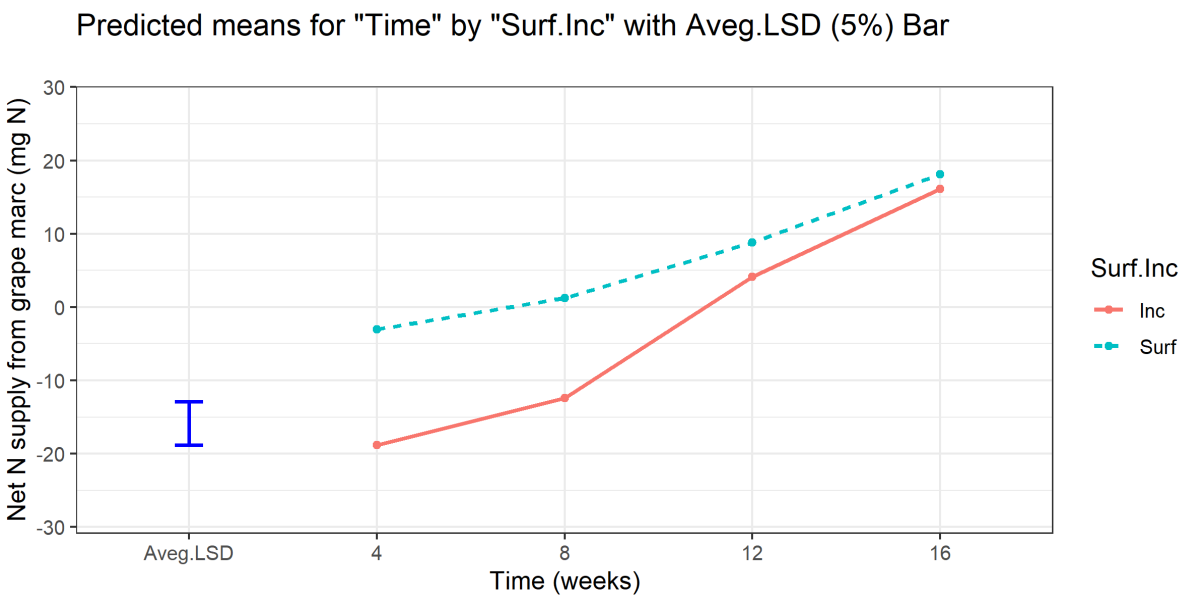

Figure 9. Net nitrogen supply from surface or incorporated grape marc over four sequential sampling points.

Figure 9 presents the net N supply from the surface or incorporated marc over the four time points (4, 8, 12, 16 weeks). This does not separate out the effect of marc rate. The incorporated marc treatments showed significantly different net N supply at each of the four time points. However, at the 4- and 8-week time points the incorporated marc showed negative net N supply (immobilisation), at 12 weeks close to no net N supply and at 16 weeks positive net N supply. The surface marc also displayed a trend of increasing net N supply at the sequential time points. However, at 4 and 8 weeks the net N supply was close to zero, but at 12 and 16 weeks the net N supply was significantly higher.

3.5.3 2-way interaction: time by Marc Rate

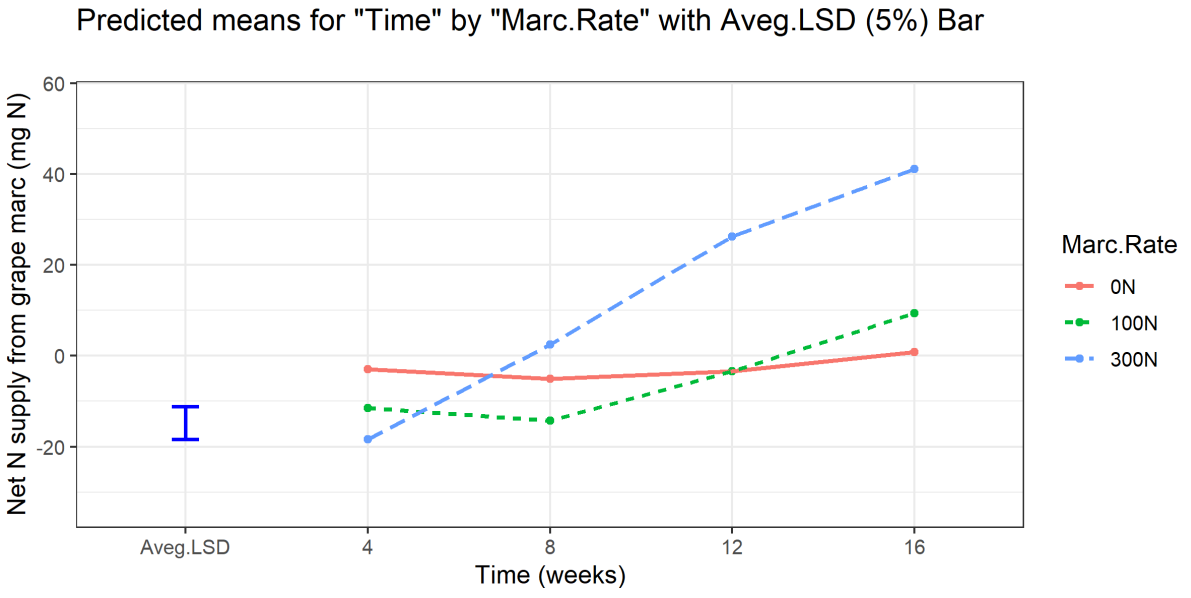

Figure 10. Net nitrogen supply from three rates of grape marc over four sampling points.

There was no significant difference in the net N supply from the 0N treatments over the four sampling points, from 4 to 16 weeks (Figure 10). The 100-kg N treatments gave rise to immobilisation of N at the 4-, 8- and 12-week sampling points and only a slight positive N supply at 16 weeks. The 300-kg N treatment gave rise to significantly increasing net N supply over the four sequential time points. However, at the 4-week time point there was significant N immobilisation, and neutral N supply at the 8-week sampling point. It was not until the 12- and 16-week sample points that the 300-kg N treatment showed positive net N supply.

3.5.4 2-way interaction: Surface or Incorporated Marc by Marc Rate

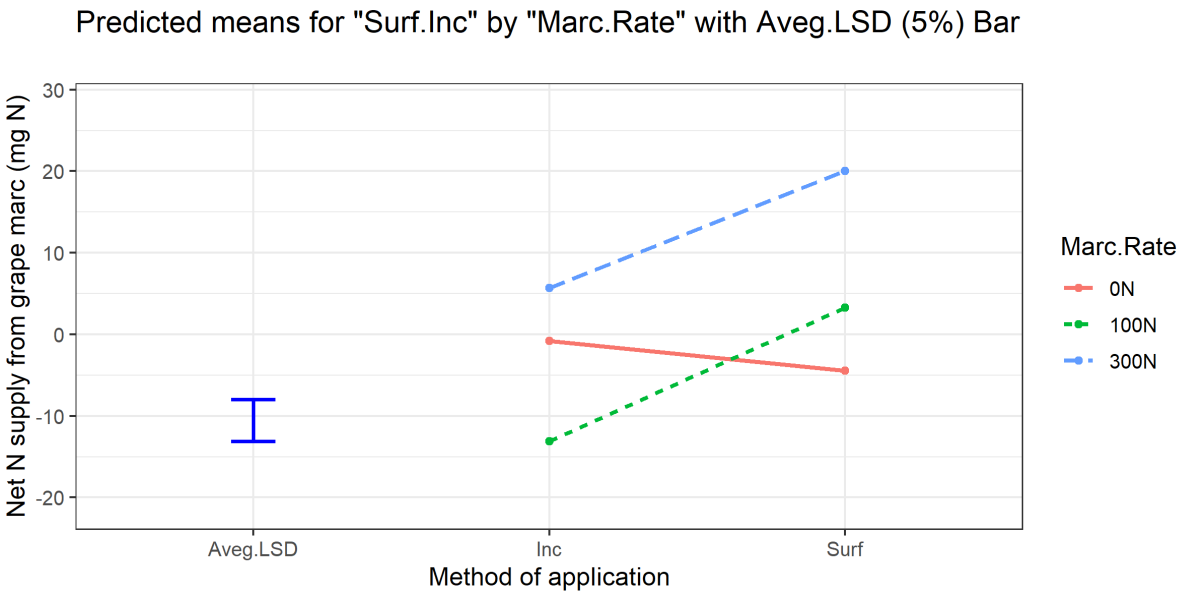

Figure 11. Net nitrogen supply from three rates of grape marc either applied to the soil surface or incorporated into the soil.

There was no significant difference in the net N supply from the 0N incorporated treatment and the 0N surface treatment (Figure 11). This was to be expected as both treatments are the same. The net N supply from the 300 kg N treatment was significantly higher than from the 100 kg N treatment. This is also as you would expect; the more N supplied, the more that is available. The net N supply from surface-applied grape marc is significantly higher than from incorporated marc, i.e. more N is immobilised when marc is incorporated in the soil. The 100-kg incorporated N treatment gave rise to significant immobilisation of N, while the 100-kg surface N treatment only supplied a small amount of N. The 300-kg incorporated N treatment supplied a small amount of N, while the 300-kg surface N treatment supplied significantly more N.

3.5.5 Surface or Incorporated Marc by Marc Rate by Time

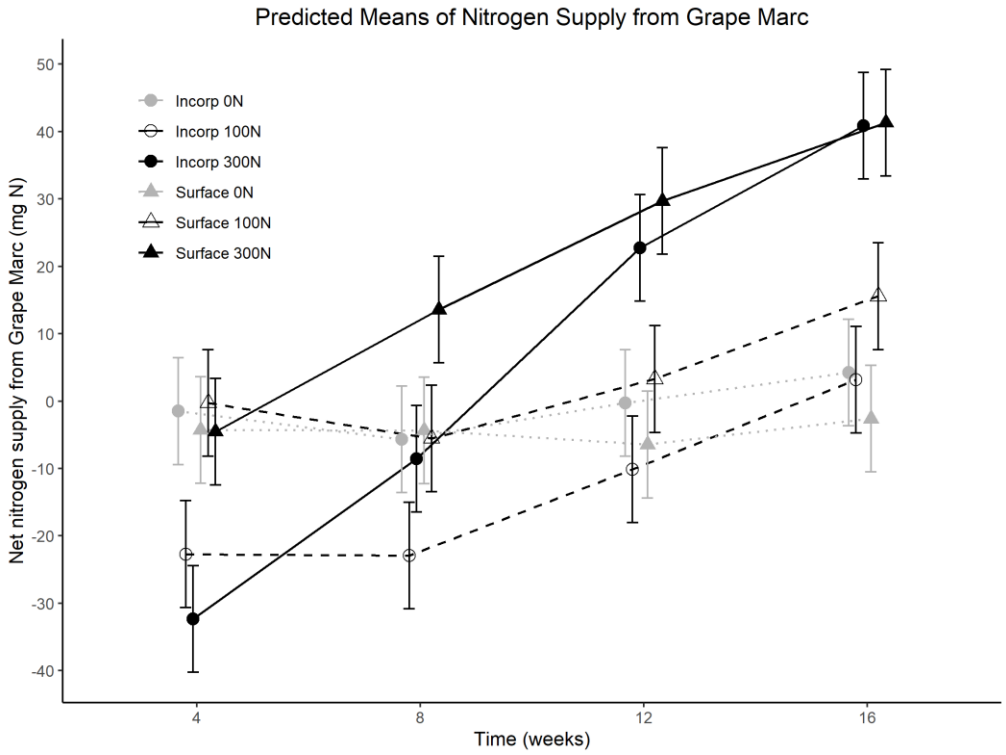

Figure 12. Net nitrogen supply from three rates of grape marc applied to the soil surface or incorporated into the soil at four sequential time points following application of marc (bars in the plot are 95% confidence intervals).

Figure 12 displays the 24 individual treatment means; i.e. 3 marc rates (0, 100, 300 kg) * 2 application methods (surface & incorporated) * 4 time points (4, 8, 12, 16 weeks). Each mean is derived from three replicate pots.

The data points in the graph indicate that there was little or no net N supply from the ON and 100N treatments at all four sample points. In fact, all treatments predominantly exhibited net N immobilisation at the 4- and 8-week time points. The only exception was for the 300-kg surface-applied N treatment at 8 weeks, where there was some positive N supply at 8 weeks. The 300N surface and incorporated treatments at the 12- and 16-week sample points exhibited a positive net N supply.

3.6 Proportion of nitrogen supplied in the grape marc that became available

One of the important considerations when applying grape marc to land is how quickly the N supplied in the grape marc actually becomes available for plant use. This will assist in determining the time of application of the marc and the amount that is likely to be taken up by plants in the vineyard and ultimately what the risk is of losing N from the land to which it is applied.

The initial analyses of the grape marc (Table 2) determined the average N content and from these analyses we calculated the amount of grape marc that needed to be applied per pot in order to be

applying the equivalent of 100 or 300 kg N/ha. The method for determining the net-N supplied from each pot was outlined in Equations 1 and 2 in Section 3.5. Knowing the amount of N supplied from the grape marc and the net-N supplied over the course of the experiment we were able to calculate the proportion of the N supplied from the grape marc that was utilised.

Greater than 90% of the N contained in the grape marc remained at the end of the experiment for all treatments with only a small proportion of the N released over the course of the 16-week experiment. The greatest amount of N supplied was from the surface-applied marc at a rate of 100 kg N/ha. However, after 16 weeks this only represented 9.6% of the N that was initially applied through the marc (Table 9). In contrast, the marc treatment that was incorporated at a rate of 100 kg N/ha still had not released any of its associated N at the end of the experiment. These results highlight that the N release from marc is likely to be much slower than anticipated and has a period where N immobilisation will most likely occur. Consequently greater time would be needed for the grape marc to be mineralised and supply N in a form that would be available for plant uptake.

The mineralisation, or decomposition, of grape marc is influenced by several factors including both the soil temperature and soil moisture content. As mentioned earlier, the average soil temperature within the pots was approximately 14.6°C, which was around 2.5°C higher than the mean annual soil temperature (10 cm depth) observed in the field in the Marlborough region. Increasing the temperature would increase the rate of mineralisation provided there was no other limiting factors. Therefore, the rate at which the marc was mineralised in this glasshouse study may be faster than what would be observed in the field (i.e. in situ). As the process of mineralisation is a microbial-mediated process, the soil moisture is another factor that can have an effect. When the soil is too wet, this process would be slowed because of a lack of oxygen and when the soil is too dry the process also is slowed because of a lack of substrate for microbial activity and insufficient movement through the soil. Overall, this study had warmer and drier conditions than what would be expected in the field, therefore the absolute rate of mineralisation of the grape marc in situ would need field validation. The relative differences in N mineralisation rates of treatments in our study are however very instructive.

Table 9. Proportion of nitrogen supplied by the grape marc that became available.

Time from sowing	Incorporated kg/ha N		Surface kg/ha N	
	100	300	100	300
4 weeks	–11.2%	–5.4%	2.1%	–0.04%
8 weeks	–9.1%	–0.5%	–0.6%	3.2%
12 weeks	–5.2%	4.1%	5.1%	6.4%
16 weeks	–0.6%	6.5%	9.6%	7.8%

The mineralisation process also depends on the initial composition of the substrate (i.e. marc in this instance). If the composition has a large C:N ratio, there will be a large N requirement of the microbes in order to break the material down. This N is often obtained from the soil pool leading to immobilisation and a decrease in the plant available N. As the marc starts to be decomposed, the C:N ratio decreases leading to the marc becoming a source of N due to mineralisation. While the initial C:N ratio of the marc may not be considered to be too high (i.e. C:N = 23), the type of carbon within the marc would influence the microbial decomposition. Typically, grape marc (seed, skin, rachii) has high levels of polyphenols and carbon compounds that would be considered to be more resistant to microbial decomposition than compounds such as simple sugars. Therefore, the energy requirement of microbes to process these compounds (i.e. polyphenols) is greater and the demand for N (i.e. energy source) becomes higher, resulting in more immobilisation.

4 Summary of key findings

After the application of marc to soil, there is a period whereby immobilisation of N within the soil N pool occurs. This is likely due to the relatively high carbon to N ratio of the grape marc (C:N=23) compared with the soil (C:N=10.3). In order for microbial activity to break down the grape marc, thereby releasing N to the soil, they will also require an amount of mineral N to do this. If the C:N ratio is too high, then this demand of N is higher, resulting in net immobilisation of N from the soil pool, as observed in this experiment.

In general, the incorporation of grape marc to soil resulted in more immobilisation of soil N in the short-term compared with the surface-applied marc. Therefore, how the marc is applied may affect the N supply dynamics and should be further explored in the field.

Increasing the rate of grape marc applied from an equivalent of 100 kg N/ha to 300 kg N/ha increased the net N supply to the soil after 16 weeks. However, the proportion of N that was released from the marc over this period was similar between the two rates, particularly in the surface-applied treatments (8–10% of N released).

The immobilisation of N, observed following addition of marc to soil, will need to be carefully considered if marc is applied for the purpose of supplying N to a crop. Immobilisation of N will remove N from the plant-available pool, leading to potential N deficiencies. If the N supply is too slow (due to a period of immobilisation) then the time where nitrogen is supplied to soil may be at a period where plant uptake is slower, or at a time of year where losses due to leaching (i.e. winter/high rainfall) are greater. However, the immobilisation of N in the short term, immediately after application, may prevent losses of N during high-risk periods (e.g. winter) but the long-term nitrogen supply needs to be explored. The year-on-year dynamics of marc application also need to be explored given the yearly generation of marc material.

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