

## Subsoil nitrogen mineralisation and its potential to contribute to $\text{NH}_4$ accumulation in a Vertosol

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### Abstract

High concentrations of  $\text{NH}_4^+$  (up to 270 kg N/ha) have been observed in a Vertosol below 1 m depth in south-east Queensland. This study examined the possibility that mineralisation associated with the removal of native vegetation (*Acacia harpophylla*) for cropping was responsible for the production of  $\text{NH}_4^+$ . Particularly, the potential contribution of decomposing root material and/or dissolved organic nitrogen (DON) leached into the subsoil after clearing was investigated. The amount of N that was contained within native vegetation root material was determined from an area of native vegetation adjacent to the cleared site containing elevated  $\text{NH}_4^+$  concentrations. In addition, the amount of  $\text{NH}_4^+$  that could be mineralised in the native vegetation soil was determined by monitoring  $\text{NH}_4^+$  concentrations over 360 days in intact cores, and by conducting waterlogged incubations. To determine the rate at which a source of DON leached into the subsoil would mineralise, soil was amended with glutamic acid at a rate of 250 mg N/kg and placed under waterlogged incubation. The possibility that the acidic pH of the subsoil, or the lack of a significant subsoil microbial population, was inhibiting mineralisation was also examined by increasing soil pH from 4.4 to 7.0, and inoculating the subsoil with surface soil microorganisms during waterlogged incubations. Low concentrations of N, approximately 90 kg N/ha between 1.2 and 3 m, were found in the native vegetation root material. In addition, no net N mineralisation was observed in either the extended incubation of intact cores or in the control samples of the waterlogged incubations. Net N mineralisation was also not detected when the subsoil was amended with a source of organic N. Results indicate that this lack of mineralisation is largely due to pH inhibition of the microbial population. It is concluded that the mineralisation of either *in situ* organic material, or DON transported to the subsoil during leaching events, is unlikely to have significantly contributed to the subsoil  $\text{NH}_4$  accumulation at the study site.

*Additional keywords:* subsoil  $\text{NH}_4$ .

### Introduction

In a Vertosol soil in south-east Queensland, high concentrations of exchangeable ammonium ( $\text{NH}_4^+$ ) have been observed at depths below 1.2 m. Between 1.2 and 3 m, total amounts of  $\text{NH}_4$ -N in the order of 200–270 kg N/ha have been recorded (Hossain *et al.* 1996). Given that nitrogen (N) is often one of the most limiting nutrients for plant growth, this large reserve of  $\text{NH}_4^+$  would represent a valuable resource to the agricultural sector if it could be moved into the surface layers of the soil profile through, for example, the incorporation of deep-rooted species into cropping rotations. However, in order to be able to successfully access and manage the subsoil  $\text{NH}_4^+$  at this site, it is important to understand why the  $\text{NH}_4^+$  accumulates. This will allow identification of other sites that may contain high concentrations of  $\text{NH}_4^+$  in the subsoil, and will allow predictions of whether the  $\text{NH}_4^+$  at this site is likely to remain stable or increase/decrease over time.

The major pathway of  $\text{NH}_4^+$  formation in soil environments is usually from the mineralisation of organic N (Tate 2000). However, organic N concentrations within the area of the profile where  $\text{NH}_4^+$  accumulations exist at this site are very low—in the order of 0.01 to 0.02% (Page *et al.* 2002). Consequently, it would seem unlikely that sufficient organic

material would be present in the subsoil to account for the  $\text{NH}_4^+$  accumulation observed. However, one important feature of the subsoil  $\text{NH}_4^+$  at Warra is that it appears under areas of cultivation, but not adjacent areas of native vegetation (Page *et al.* 2002). This would suggest that the clearance of native vegetation has induced some change in the landscape that has led to the formation of  $\text{NH}_4^+$ . For this reason, it is considered possible that N mineralisation may have contributed to the  $\text{NH}_4^+$  accumulation at depth. It should be noted that previous studies of this site have not detected nitrification in the area of  $\text{NH}_4^+$  accumulation (Page *et al.* 2002), indicating that any  $\text{NH}_4^+$  formed via a mineralisation pathway would accumulate over time.

The removal of deep-rooted native vegetation and its replacement with shallow-rooted crops and pastures would have changed the soil environment. This would have killed any root material previously associated with the native vegetation, decreased nutrient extraction from deeper layers, and increased water movement through the soil profile. These changes could have triggered some  $\text{NH}_4^+$  production due to the decomposition of native vegetation root material, and because the increased water movement through the profile may have increased the leaching (and subsequent mineralisation) of dissolved organic nitrogen (DON) into the subsoil.

Other studies of agricultural subsoils have observed that the mineralisation of root material can contribute to subsoil  $\text{NH}_4^+$  production (Hadas *et al.* 1989; Weier and MacRae 1993). In addition, live roots of the Brigalow (*Acacia harpophylla*) plant (the dominant native vegetation originally present on the site) have been observed to penetrate to 4 m depth (Tunstall 1973). Consequently, it is possible that at the time of clearing root material was present where  $\text{NH}_4^+$  has accumulated, and upon decomposition may have contributed to subsoil  $\text{NH}_4^+$  concentrations.

It is also possible that the increase in water movement through the soil profile may have increased leaching of DON, and thus further contributed to subsoil organic N concentrations. Dissolved organic nitrogen will most readily leach through soils that have high concentrations of DON in the surface soil, where there is low demand for DON by surface soil microorganisms, and where soils have a sandy texture and thus limited ability to retain DON against leaching (Murphy *et al.* 2000). The surface soil horizon of the study site (0–0.1 m) has a high clay content (56%), low total N concentration (0.072%) (Dalal *et al.* 1995), and is unfertilised. Consequently, the amount of DON that leaches through to the subsoil each year is expected to be small. However, the possibility that some DON is leached each year, and that over time (e.g. the 65 years since the site has been cleared) small amounts could be mineralised and contribute to the observed accumulation of  $\text{NH}_4^+$  in the subsoil, cannot yet be discounted.

The objectives of this work were thus to determine whether mineralisation of organic N, and, in particular, the decomposition of root material or leached DON, had the potential to contribute to subsoil  $\text{NH}_4^+$  concentrations at the study site.

## Materials and methods

### *Study site*

The study site was located near Warra in southeast Queensland (26°47'S, 150°53'E). The site, which was originally under Brigalow vegetation, was cleared during the mid 1930s and has been used for dryland agriculture, predominantly wheat cropping (*Triticum aestivum* L.), ever since. The soil has been classified as a thermic, Typic Chromustert (Dalal *et al.* 1995). Relevant site characteristics for areas of native vegetation and cultivation are summarised in Table 1. The area of cultivation was in fallow when sampled. It should be noted that regular application of N fertiliser has not occurred at this site.

**Table 1. Summary of site characteristics for areas of cropping and native vegetation**

Depth (m)	NO <sub>3</sub> -N (mg/kg)		NH <sub>4</sub> -N (mg/kg)		Organic C (%)		Organic N (%)		pH		EC (dS/m)	
	Native	Crop	Native	Crop	Native	Crop	Native	Crop	Native	Crop	Native	Crop
0–0.3	1.2	28.5	1.72	2.50	0.9	0.38	0.10	0.05	7.9	8.8	0.4	0.2
0.3–0.6	<0.5	8.1	<0.5	<0.5	0.33	0.23	0.04	0.03	8.4	8.6	1.2	0.5
0.6–0.9	<0.5	8.1	<0.5	<0.5	0.25	0.16	0.03	0.02	7.7	7.9	1.5	0.5
0.9–1.2	<0.5	6.2	0.6	2.7	0.25	0.18	0.02	0.02	4.9	5.6	1.5	0.8
1.2–1.5	<0.5	4.4	0.5	14.2	0.18	0.18	0.02	0.01	4.5	4.9	1.5	1.0
1.5–1.8	<0.5	2.5	0.5	18.9	0.13	0.15	0.01	0.01	4.4	4.5	1.6	1.1
1.8–2.1	<0.5	<0.5	0.9	16.1	0.11	0.15	0.01	0.01	4.3	4.4	1.6	1.3
2.1–2.4	<0.5	<0.5	0.5	11.4	0.14	0.11	0.01	0.01	4.3	4.5	1.7	1.6
2.4–2.7	<0.5	<0.5	0.5	8.9	0.12	0.11	0.01	0.01	4.3	4.4	1.7	1.6
2.7–3.0	<0.5	<0.5	0.9	4.9	0.09	0.12	0.01	0.01	4.2	4.3	1.7	1.7

#### Soil sampling and processing

Soil sampling was conducted on 2 adjacent land management units, cropping and native vegetation. Samples were taken to a depth of 3 m using a hydraulically operated soil sampler that extracted cores of 0.042 m diameter to 1.5 m, and 0.039 m diameter to 3 m. Sampling was conducted at 5 separate points within each land management unit. Cores were sampled at regular intervals along a 10-m transect on the cropping site, and along a 100-m transect on the native vegetation site. The difference in transect length on the native vegetation site was due to difficulty in finding spaces suitable for vehicular access within a 10-m distance. It should be noted that previous sampling of this site has indicated that NH<sub>4</sub><sup>+</sup> occurs relatively uniformly across the area of cultivation, and that a 10-m distance was considered sufficient to obtain representative samples in this area. Once collected, all soil cores were divided into 0.3-m sections, placed in plastic bags, and transported back to the laboratory for processing.

#### Quantification of in situ mineralisable-N

##### Root measurement

To estimate the amount of N contained within native vegetation root material, roots were washed from 5 separate soil profiles collected across the native vegetation site (as described above), dried in a fan-forced oven at 60°C, and weighed. The average weight of roots collected was then used in combination with the total N content of this material (determined as described below), to estimate the total quantity of N contained in roots at 0.3-m depths throughout the soil profile. To provide a comparison between the amount of N contained in root material and the amount of exchangeable NH<sub>4</sub><sup>+</sup> present in cleared areas, analysis of exchangeable NH<sub>4</sub><sup>+</sup> concentrations down to 3 m depth was also conducted at the 5 sites sampled throughout the area of cropping.

##### Intact core incubation

To examine the mineralisation rate of *in situ* organic material under conditions similar to those observed in the field, intact cores were collected and incubated for a period of 360 days. Cores were taken from the 1.8–1.9 m depth of the soil profile at the 5 sampling points across the native vegetation site. To provide enough cores to enable sampling at various times throughout the period of incubation, 4 cores taken were within 0.10 m of one another at each of the sampling points, to be used for destructive sampling at 0, 40, 120, and 360 days. Once collected, intact cores were randomly placed into 3 sealed incubation jars that were linked in series and flushed with high purity N<sub>2</sub> gas. Flushing was conducted to ensure that cores were kept in a low oxygen environment, similar to what was expected at depth in the soil. Jars were kept in a constant temperature room at 22°C. At 0, 40, 120, and 360 days after sampling, 1 core from each of the 5 replications was destructively sampled and analysed for exchangeable NH<sub>4</sub><sup>+</sup> and nitrate as described below.

##### Waterlogged incubation

A waterlogged incubation was used to assess the quantity of potentially mineralisable N present in the subsoil at the time of clearing. The soil used for this incubation was obtained from the 1.5–3 m layer of the

profile at the native vegetation site. This section of the profile was chosen for analysis as it represented the area of maximum  $\text{NH}_4^+$  accumulation in the area of cultivation, and was large enough to provide sufficient soil material for the experiment. Waterlogged incubations were conducted as described in Waring and Bremner (1964), except that soil was incubated at 40°C for 1 week. As a part of this incubation, a series of treatments were also applied in a 2-level factorial design (i.e. present/absent) to examine environmental factors that may limit net mineralisation. These treatments involved increasing soil pH from 4.4 to 7.0 ( $\pm 0.5$ ) with  $\text{CaCO}_3$  to determine if soil acidity was inhibiting soil microorganisms, and inoculating the subsoil with surface soil microorganisms, as described by Rovira and Vallejo (1997), to determine if there was a sufficiently large microbial population at depth to mineralise organic N. Five replicates were used for each treatment. After incubation, the  $\text{NH}_4^+$  concentration of samples was determined as described below. Samples of unincubated soil were also extracted at this time to provide a comparison of  $\text{NH}_4^+$  concentrations between incubated and un-incubated samples.

#### *Mineralisation of added organic N*

To determine the capacity of the subsoil to mineralise a source of DON, such as may be leached into the subsoil, a second waterlogged incubation was conducted. The procedures used during this experiment were as described above, except that soil from the cropping site was used instead of soil from the native vegetation site, given that this is the site where any mineralisation of leached DON would occur. During this experiment the amount of  $\text{NH}_4^+$  produced by control soil placed under waterlogged incubation was compared with that produced by the soil when it had been amended with an easily mineralisable source of organic N (glutamic acid,  $\text{C}_5\text{H}_9\text{NO}_4$ ) at the rate of 250 mg N/kg soil. Treatments to increase soil pH and inoculate the subsoil (administered as described previously) were also included in this experiment to determine the effect that these factors had on the net mineralisation of the added organic N.

#### *Analytical methods*

Total N in root material was determined using a Kjeldahl digestion, and the  $\text{NH}_4^+$  produced during this reaction measured colorimetrically (Crooke and Simpson 1971). Exchangeable  $\text{NH}_4^+$  and nitrate were extracted from samples by shaking soil for 1 h in a 2 M KCl solution [1:4 soil:solution ratio for soil sampled to characterise  $\text{NH}_4^+$  concentrations at the cropping site, and for intact cores (Buresh *et al.* 1982); and 1:10 soil:solution ratio for waterlogged incubation samples]. Those samples that had not been amended with organic N were then analysed for  $\text{NH}_4^+$  colorimetrically, using a method based on the indophenol blue technique (Henzell *et al.* 1968). Nitrate in extracts was measured by reducing nitrate to nitrite with hydrazine and a copper catalyst, and measuring the total nitrite produced using the Greiss-Ilosvay reaction as described by Bremner (1965). The  $\text{NH}_4^+$  in those samples amended with organic N was measured using steam distillation with MgO, due to interference of glutamic acid with the colorimetric technique (Bremner and Keeney 1966).

#### *Statistical analysis*

One-way ANOVA was used to analyse differences in the  $\text{NH}_4^+$  concentration of intact cores, differences between unincubated samples and those placed under waterlogged incubation, and differences between control samples and those amended with organic N. The effect of soil environmental characteristics on mineralisation during the waterlogged incubation study were analysed using a  $2 \times 2$  factorial analysis (i.e. pH  $\times$  inoculation). Analyses were conducted using the SAS statistical package (SAS 1999), and differences considered significant if  $P < 0.05$ .

## **Results**

### *Quantification of in situ mineralisable N*

#### *Root measurement*

The average concentration of N contained in brigalow root material between 0 and 3 m on the native vegetation site is presented in Table 2. Exchangeable  $\text{NH}_4^+$ -N concentrations recorded in the cropped soil are also presented in this table. These results show that the amount of N contained in root biomass was highest in the 0–0.3 m layer of the profile, and then steadily decreased with increasing depth, to concentrations  $< 5$  mg N/kg soil below 1.8 m depth. The amount of N that was contained in brigalow root material between 1.2 and

**Table 2. Total N concentration of brigalow root material from the native vegetation site, and the exchangeable  $\text{NH}_4$ -N concentration of soil from the cropping site between 0 and 3 m**

Standard errors are presented in parentheses

Depth (m)	Root-N (mg N/kg)	$\text{NH}_4$ -N (mg N/kg)
0–0.3	159.1 (23.9)	2.5 (0.3)
0.3–0.6	21.3 (1.9)	0.5 (0.12)
0.6–0.9	11.5 (1.5)	0.4 (0.05)
0.9–1.2	12.2 (5.8)	2.7 (0.92)
1.2–1.5	4.2 (0.5)	14.2 (2.45)
1.5–1.8	5.6 (1.0)	15.0 (1.28)
1.8–2.1	2.8 (0.5)	18.9 (0.92)
2.1–2.4	2.0 (0.6)	16.1 (0.71)
2.4–2.7	2.2 (0.3)	11.4 (0.71)
2.7–3.0	3.5 (1.1)	8.9 (0.81)

3 m (the area of subsoil  $\text{NH}_4^+$  accumulation) was approximately 90 kg N/ha. The exchangeable  $\text{NH}_4^+$ -N concentrations in the cropped soil were low to 1.2 m depth, and then increased to a maximum of 18.9 mg N/kg at 1.8–2.1 m. Concentrations then decreased slightly, but remained elevated to 3 m [ $\text{NO}_3^-$ -N concentrations in the area of exchangeable ammonium accumulation were negligible, <5 mg N/kg (Table 1)]. The amount of exchangeable  $\text{NH}_4$ -N between 1.2 and 3 m was approximately 380 kg N/ha.

#### *Intact core and waterlogged incubations*

There was no significant increase ( $P > 0.05$ ) in the  $\text{NH}_4^+$ -N concentration of intact cores during the 360-day period of the experiment. Nitrate concentrations for all samples measured were also negligible.

Results from the waterlogged incubation showed that there was no significant increase ( $P > 0.05$ ) in the  $\text{NH}_4^+$ -N concentration of samples subjected to waterlogging compared with unincubated samples. Increasing the soil pH resulted in a significant increase ( $P < 0.05$ ) in  $\text{NH}_4^+$ -N concentration (to approximately 3.6 mg N/kg), but the alteration of the microbial population had no significant effect ( $P > 0.05$ ) on  $\text{NH}_4^+$ -N production. It should be noted that the addition of inoculum to samples had no detectable effect on soil pH.

#### *Mineralisation of an added organic N source*

Subsoil samples incubated in the presence of organic N (without alteration of soil pH or microbial population) showed no significant increase ( $P > 0.05$ ) in  $\text{NH}_4^+$ -N concentration relative to control samples (Table 3). However, when organic N was added in combination with a pH increase and/or inoculation treatments, a significant increase ( $P < 0.05$ ) in  $\text{NH}_4^+$  concentration was observed. The greatest amount of  $\text{NH}_4^+$  was produced when pH and inoculation treatments were used in combination (Table 3).

## **Discussion**

The quantity of organic N contained within root biomass was low, and results indicate that the decomposition of root material is only capable of supplying a small amount of exchangeable  $\text{NH}_4^+$ -N. In the 1.8–2.1 m section of the soil profile at the native vegetation site, for example, an average of 2.8 mg N/kg was contained in root biomass (Table 2). In

**Table 3.  $\text{NH}_4$  concentration (mg-N/kg) of soil from a cropping site (1.5–3 m) incubated with added organic N (ON) in combination with inoculum (I) and increased pH treatments**

Means followed by the same letter are not significantly different ( $P > 0.05$ )

Treatment	$\text{NH}_4$ (mg N/kg)
Control	9.0a
ON	8.5a
ON/I	23.1b
ON/pH	31.8c
ON/I/pH	227.6d

this same section of the soil profile on the cropping site, however, an average exchangeable  $\text{NH}_4^+$ -N concentration of 18.9 mg N/kg was observed (Table 2). From this it is clear that even if all the N contained within root material under native vegetation were to be released into the  $\text{NH}_4^+$ -N fraction, it would not be capable of supplying the quantity of  $\text{NH}_4^+$ -N observed in cleared areas. This indicates that the death and subsequent decomposition of root material upon clearing of the native vegetation is unlikely to have been a major contributor to subsoil  $\text{NH}_4^+$ -N concentrations.

The above result was further confirmed by the failure to observe any net mineralisation in either the extended incubation of intact cores, or when samples were placed under waterlogged incubation (without alteration of soil pH or microbial population). This result indicates that not only is the quantity of organic N in the subsoil of this site low, but that net mineralisation of this material is undetectable. It is not unusual to observe low rates of net mineralisation in subsoil environments. For example, one study examining mineralisation in intact cores from the 1.10–1.15 m layer of a pasture soil failed to observe any net mineralisation over 84 days (Weier and MacRae 1993). However, there are studies that have observed active net mineralisation in subsoil environments (Hadas *et al.* 1989; Weier and MacRae 1993), and sometimes this has been observed to occur as deep as 2.3 m (Swensen and Bakken 1998). Consequently, the fact that the soil under study is a subsoil should not in itself be a reason for an absence of net mineralisation. This indicates that there is likely to be some environmental factor/s preventing net mineralisation at this site.

The significant increase in  $\text{NH}_4^+$ -N concentration observed during waterlogged incubations when soil pH was increased from 4.4 to 7.0 indicates that subsoil acidity is at least partly responsible for the slow rate of net mineralisation at this site. The depression of mineralisation by soil acidity, and increases in net mineralisation after liming has been observed in a number of studies (Fu *et al.* 1987; Sapek 1997; Curtin *et al.* 1998). There are generally 2 explanations put forward to account for the stimulatory effect of increased pH. The first is that liming eliminates the  $\text{H}^+/\text{Al}^{3+}$  toxicity and/or  $\text{Ca}^{2+}$  deficiency that inhibit the microbial biomass in an acidic soil (Adams and Martin 1984). The second explanation is that an increase in pH can increase the availability of substrate for mineralising organisms by increasing the amount of soluble organic material (Curtin *et al.* 1998).

In the current study, it is believed that the increase in  $\text{NH}_4^+$ -N production as a result of liming is due to the removal of pH toxicity, not due to an increase in mineralisable substrate. Even when a large amount of readily mineralisable organic N was added to

this subsoil (in the form of glutamic acid), no significant increase ( $P > 0.05$ ) in  $\text{NH}_4^+$ -N concentrations could be detected (Table 3). It was not until the soil pH was increased and/or surface soil microorganisms were added to the soil that any net mineralisation of the added organic N occurred. This result indicates that the ammonifying organisms in the subsoil are severely inhibited by the acidic conditions at depth, and are not able to undertake net mineralisation even when there is an abundance of a readily mineralisable substrate present.

The observed limitation of N mineralisation by low pH is consistent with previous studies conducted at this site where nitrifying activity was examined (Page *et al.* 2002). In these studies the nitrification rate in the subsoil was found to be extremely low (undetectable over a 180-day period), and to be limited by the lack of an active nitrifying population (Page *et al.* 2002). The low soil pH was also found to prevent nitrification even if the subsoil was inoculated with organisms present in the surface of the profile, indicating that acidity may have prevented nitrifiers colonising the subsoil (Page *et al.* 2002). The results of this and the current study clearly indicate that the pH of the subsoil environment is inhibitory to several groups of microorganisms important in the N cycle.

It should also be noted, however, that the failure to observe significant amounts of net mineralisation in the subsoil might also be partly related to an inherently low mineralisation activity in the subsoil microbial population. The increase in  $\text{NH}_4^+$ -N concentration that was observed when organic-N was added to the subsoil in combination with inoculum (Table 3) demonstrates that surface soil microorganisms were capable of carrying out some net N mineralisation in the subsoil despite the acidic conditions. In addition, when the pH of the subsoil was increased, the amount of  $\text{NH}_4^+$ -N produced by those treatments containing inoculum was nearly 5 times greater than those without inoculum (Table 3). This indicates that the subsoil microbial population has a somewhat lower capacity for mineralisation than surface soil organisms. This reduced capacity could be due to lower total numbers of mineralising organisms in the subsoil, or due to the presence of a less active microbial population. Regardless, these data indicate that the ability of the indigenous subsoil microbial population to conduct net mineralisation is severely limited, and that no organic N, whether it is present *in situ* or transported to the subsoil during leaching events, is likely to be converted to  $\text{NH}_4^+$ -N at a rate great enough to account for the accumulation of  $\text{NH}_4^+$ -N observed in the subsoil.

Since this study has clearly failed to identify the source of the subsoil  $\text{NH}_4^+$ -N at the Warra site, further hypotheses regarding the mechanism of  $\text{NH}_4^+$ -N formation need to be identified. Because it is known that the clearance of native vegetation is in some way linked to the  $\text{NH}_4^+$ -N build-up, future hypotheses should focus on the relationship between  $\text{NH}_4^+$ -N and the changes in the soil environment that would have occurred after clearing. For example, the release of  $\text{NH}_4^+$ -N from fixed fractions (i.e. ammonium trapped in the interlayer of clay minerals) may be advanced as a hypothesis, given that after clearing any  $\text{NH}_4^+$ -N produced via this pathway would have been able to accumulate in an exchangeable form due to an absence of nutrient extraction by deep-rooted tree species. Nitrate reduction to  $\text{NH}_4^+$ -N (via either biotic or abiotic pathways) may also have occurred during periods of transient anaerobiosis, which may have developed due to increased subsoil moisture contents after clearing. Similarly, the gradual leaching of  $\text{NH}_4^+$ -N into the subsoil over time in response to increased water movement through the soil profile is also a possible pathway of  $\text{NH}_4^+$ -N formation. The possible contribution of each of these pathways to subsoil  $\text{NH}_4^+$ -N concentrations will be investigated in future studies.

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